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Electrocatalytic reduction of metronidazole using titanocene/Nafion®-modified graphite felt electrode

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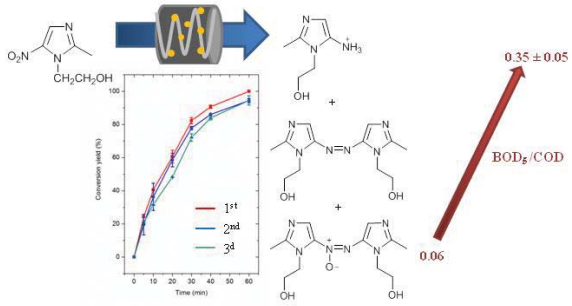
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Graphical Abstract



ABSTRACT

The main objective of this study was to examine the feasibility of an electrocatalytic reduction on titanocene/Nafion®-modified graphite felt electrode, as pretreatment, before a biological treatment, for the degradation of metronidazole, a nitro biorecalcitrant pollutant. A titanium complex, know as an effective catalyst in the reduction of nitro groups, was immobilized on the electrode surface by encapsulation into a Nafion® film. The different operating conditions used to prepare the modified electrode, *i. e.* the initial concentrations of catalyst and Nafion® and the sonication time, were optimized and the modification of the electrode was highlighted by cyclic voltammetry and electronic scanning microscopy coupled with energy dispersive spectroscopy analysis. The results show a good stability and reproducibility of the modified electrode. Flow heterogeneous catalytic reduction of metronidazole was then carried out with the titanocene/Nafion®-modified graphite felt as working electrode. The HPLC analysis underlined the total reduction of metronidazole after 1 hour and the evolution of the biological oxygen demand to chemical oxygen demand ratio showed a significant increase of biodegradability from 0.06 before pretreatment to 0.35 ± 0.05 after electrolysis on the modified graphite felt electrode. The comparison of both homogeneous and heterogeneous reactions underlined the interest of the immobilization process that led to a higher stability of the catalyst, giving rise to a higher turnover number and an improvement of biodegradability.

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The stability of the modified electrode was investigated after electrolysis by cyclic voltammetry and successive electrolyses.

Keywords: Modified electrode; Electrocatalytic reduction; Graphite felt; Titanocene; Metronidazole

1. Introduction

In recent years, the combined processes, in particular physicochemical and biological combined treatments, have received a growing attention for their efficiency to achieve complete mineralization of biorecalcitrant compounds with the objective to reduce operating costs [1-12]. In such systems, a physicochemical pretreatment such as an advanced oxidation process (AOP) or an electrochemical method is used to change the structure of pollutants. The aim is to obtain less toxic and more biodegradable intermediates, giving rise to a complete mineralization after a subsequent biological treatment. A lot of studies have highlighted the possibility to use combined processes for the removal of non-biodegradable pollutants such as pesticides [1, 4-6, 9, 11, 13-15], dyes [7, 10] and pharmaceuticals [3, 8, 12, 16-19].

Electrochemical pretreatments are particularly interesting since the reduction or oxidation of a product can be performed without the addition of chemical reducing or oxidizing agents that can be pollutants. A lot of them are AOPs that lead to the formation of hydroxyl radicals. Due to their high reactivity, these processes are not selective and can lead to by-products that can be more toxic than the target compounds. To achieve an effective, economical, low-energy and well-controlled electrochemical process, direct oxidation or reduction have also been considered when the target compound is electroactive. Advantageously, these methods are more selective than AOPs and rarely lead to total mineralization, which is particularly interesting for a coupling with a biological treatment. Several studies on the use of these methods to achieve the pretreatment of recalcitrant pollutants have been carried out. They have shown their efficiency for the removal of several recalcitrant molecules such as phosmet an organophosphorous insecticide [14], a chlorinated phenoxy herbicide 2,4-dichlorophenoxyacetic acid [6, 9, 13] and extensively used antibiotics such as sulfamethazine [19] and tetracycline [17].

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Electrocatalysis in the presence of a catalyst has been used to achieve high selective electrochemical processes and to reduce energy consumption by performing electrochemical reduction at less cathodic potentials. For example, the coupling of a pretreatment with a nickel -modified graphite felt electrode and a biological treatment for the removal of a biorecalcitrant antibiotic, tetracycline, has been carried out [12]. Tetracycline can be oxidized in alkaline conditions on nickel at an oxidation potential of 0.5 V/SCE, which is significantly lower than with a graphite felt electrode (1 V/SCE). A good biodegradability of the solution after oxidation on the Ni-modified electrode (biological oxygen demand to chemical oxygen demand ratio: $BOD_5/COD = 0.46$) was obtained with a significant decrease in toxicity. These results were confirmed during the biological treatment with activated sludge culture, leading to a 69% overall TOC decrease during the combined process.

The dehalogenation reaction in the presence of $Ni(tmc)Br_2$ complex, of a chlorinated compound, 1,3-dichloropropane, that has been detected as contaminant in source water, has also been accomplished [20]. Flow heterogeneous catalytic reduction of 1,3-dichloropropane with the catalyst immobilized on the electrode, has shown a total dehalogenation in only 3.5h and a dechlorination yield of 80%. Comparison with the catalyst in solution has highlighted a better stability of the immobilized catalyst with higher turnover number and current efficiency than in homogeneous solution.

More recently, we reported the interest of a titanium complex for the electrocatalytic reduction of metronidazole (MNZ), a biorecalcitrant antibiotic [18]. Metronidazole contains a nitro group that is known to reduce the biodegradability of the molecule. The electroreduction of metronidazole in the presence of titanocene dichloride ($(C_5H_5)_2TiCl_2$), known as catalyst for the selective reduction of nitro groups into amino compounds, increased the biodegradability of the compound allowing a rapid and efficient subsequent biological treatment: 85% of total organic carbon was removed by means of the combined process. These results emphasized the interest of indirect electrolysis involving the reduction of nitro groups into amino compounds as pretreatment before a biological treatment.

However, the presence of the catalyst in solution seems to affect the trend of biodegradability. In addition, the electrocatalytic reduction of the nitro group is not as selective as expected when the catalyst is in solution due to a competition with the direct reduction of metronidazole on the electrode.

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To overcome the drawbacks of homogeneous catalysis and improve the electrocatalytic activity towards the reduction of metronidazole, the immobilization of the titanium complex onto the electrode is envisaged in this work. Furthermore, the immobilization of the catalyst onto the working electrode makes the indirect electrolysis even more attractive. Firstly, the catalyst is thus located at the interface, place of the electron transfer reactions. Secondly, the electrolysis process is simplified. Indeed, the problem of solubility of the catalyst in the reaction medium is avoided and the purification process and the recovery are facilitated. Thirdly, the catalyst immobilization usually improves its stability and/or selectivity compared with the catalyst in solution. The immobilization of the catalyst was achieved by polymer coatings using Nafion®, a perfluorinated anionic polyelectrolyte which exhibits permselectivity towards cations. Chemically modified electrodes were prepared by adsorption of catalyst/Nafion® films on the graphite felt electrode. The effects of various experimental parameters such as the initial concentration of catalyst, the concentration of Nafion® and the sonication time were carried out in order to optimize the preparation of the modified electrode. It was characterized by scanning electron microscopy with energy dispersive X-ray spectroscopy (SEM-EDS) analysis and cyclic voltammetry and the catalytic performances of titanocene/Nafion®-modified electrodes toward the reduction of metronidazole were then studied.

2. Experimental

2.1. Chemicals and materials

Metronidazole (MNZ) with 99% of purity and titanocene dichloride $(C_5H_5)_2TiCl_2$ were obtained from Tokyo Chemical Industry Europe. H_2SO_4 (purity 99 %) and absolute Ethanol was purchased from VWR. 5% solution of Nafion® 117 and Acetonitrile (purity 99.9%) was HPLC (high performance liquid chromatography) grade obtained from Sigma-Aldrich. Graphite felt (RVG 4000) was supplied by Mersen (France). Its specific area measured by the Brunauer, Emmett et Teller (BET) method, its volume density and its carbon content were 0.7 $m^2\ g^{-1}$, 0.088 g cm^{-3} and 99.9%, respectively.

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2.2. Materials for the electrochemical pretreatment

Electrochemical pretreatment, in continuous system, was performed in a home-made flow cell [20]. Two DSA (Dimensionally Stable Anodes) electrodes (AC-2004 ECS International Electro Chemical Services, France) (1 cm of diameter) were used as counter electrode and compartments were separated by cationic exchange membranes (Ionac 3470 – Lanxess SAS, Courbevoie, France). The reference electrode (Saturated Calomel Electrode – SCE) was positioned in the middle of the modified/fresh graphite felt (1 cm^3) and the potential control was performed using a potentiostat (Princeton Applied Research, EG&G 362). The contact with the graphite felt is ensured with a Pt wire. To ensure a good homogeneity of the potential distribution in the three dimensional working electrodes, the felt was located between the two counter-electrodes [21]. The cell was thoroughly rinsed with distilled water before and after each experiment. The solution percolated the graphite felt electrode at various flow rates monitored by a Gilson minipuls 2 peristaltic pump (Middleton, WI, USA). The electrocatalytic reduction of metronidazole (100 mg L^{-1} of MNZ in 0.5 M H_2SO_4) were carried out at -0.5 V/SCE at a flow rate of 3 mL min^{-1}.

2.3. Analytical Procedure

2.3.1. Electrochemical Analysis

The electrochemical analysis was performed at room temperature under nitrogen atmosphere to avoid dissolved oxygen using a versaSTAT3 AMETEK Model (Princeton Applied Research) potentiostat/galvanostat. Three-electrode cell containing a modified graphite felt electrode (1cm^3) fixed to a platinum wire as working electrode, saturated calomel electrode (SCE) as reference electrode and a platinum wire counter electrode were used.

2.3.2. Scanning electron microscopy and X-ray microanalysis (SEM-EDS)

This technique gives morphological (Image) and chemical (elemental composition) information of the modified electrode. It was performed with JSM-7100F Field Emission Scanning Electron microscopy equipped by EDS (Energy Dispersive X-Ray Spectroscopy) Detector SDD type (Silicon Drift Detector) X-Max 80 mm^2 Oxford Instruments

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AZtecEnergy. Accelerating voltage was 10 kV with a beam current of 1 nA and a spectra collection time of 100s.

2.3.3 High Performance Liquid Chromatography (HPLC)

MNZ concentration was determined by HPLC using a Waters 996 system equipped with waters 996 PDA (Photodiode Array Detector) and Waters 600 LCD Pump. The separation was achieved on a Waters C-18 (5 µm; 4.6 × 250 mm) reversed-phase and the mobile phase consisted of a mixture of acetonitrile / ultra-pure water (20 / 80, v / v) and 0.1% formic acid. The flow rate was set at 1 mL min^{-1} and 10 µL injections were used. Detection of MNZ was carried out at 318 nm and the retention time was approximately 4.3 min. Before HPLC analysis, the pH of the electrolyzed solutions should be raised at 3.

2.3.4 UPLC–MS / MS method

Ultra-pressure liquid chromatography

The devices used are detailed in previous works [13]. The analytes were separated by means of a Waters Acquity UPLC system (Waters corporation, Milford, MA, USA) consisting of an Acquity UPLC binary solvent manager, an Acquity UPLC sample manager and an Acquity UPLC column heater (45 °C) equipped with a Waters Acquity UPLC BEH C18 column (2.1 mm × 100 mm, 1.7 µm particle size) (Milford, MA, USA). Gradient LC elution was performed with 0.1% formic acid in acetonitrile as mobile phase A and an ultrapure water 9:1 acetonitrile (v / v) mix, with added 0.1% (v / v) of formic acid as mobile phase B. It was carried out as followed: 8 min with 0 to 100% A, 0.5 min with 100% A and 2.5 min with 100 to 0% A. Separation of the analytes on the column was performed at 0.4 mL min^{-1} flow rate.

Tandem mass spectrometry

A Quattro Premier triple-quadrupole mass spectrometer (Micromass, Manchester, UK) equipped with an electrospray ionization source was used for degradation products detection. MS / MS detection was performed in positive mode. Source conditions were capillary voltage 3 kV, source temperature 120°C and desolvation temperature 350°C. The cone and

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desolvation gas flows (N_2) were 50 and 750 L h^{-1}, respectively. The analytical device was controlled by Micromass MassLynx 4.1 software. Spectra were acquired between 80 and 500 m/z between 0 and 8 min at a cone voltage of 20 V.

2.3.5 Chemical Oxygen Demand (COD) measurements

Chemical Oxygen Demand was measured by means of a Test Nanocolor® CSB 160 from Macherey-Nagel (Düren, Germany). The amount of oxygen required for the oxidation of the organic and mineral matter at 160°C for 30 min was quantified after oxidation with $K_2Cr_2O_7$ at acidic pH and heating.

2.3.6 Biological Oxygen Demand (BOD_5) measurements

Biological Oxygen Demand measurement was carried by the test NANOCOLOR® BOD_5. This test allows a simplified determination of the biological oxygen demand of a diluted sample. The oxygen-enriched sample is incubated in test tubes for 5 days at 20±1°C in the dark. Determining the dissolved oxygen takes place after five days according to the method of Winkler EN25813-G21 by photometric evaluation of the iodine color.

2.4. Preparation of the modified electrode

The modified electrodes were prepared by adsorption of Nafion®/catalyst films on graphite felt electrode. Solutions of titanium complex in absolute ethanol were prepared at different concentrations ranging from 0.5 to 4 g L^{-1}. After complete dissolution, 5 mL of a solution were prepared from 4 to 4.6 mL of the titanocene solution and 0.4 to 1 mL of a ~5% solution of Nafion® 117, leading to a solution with Nafion® at 0.4 to 1%. Fresh graphite felt (1cm^3) was then washed with ethanol and dipped in the mixture. After sonication in ultrasonic bath for 15 min to 45 min, the felt is pressed and air-dried (Fig. 1). Before using, the modified graphite felt electrode was rinsed with 0.5 M H_2SO_4 solution.

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3. Results and discussion

3.1. Immobilization of the titanium complex on the electrode

The catalyst was immobilized on a graphite felt electrode (1 cm^3) by incorporation into a Nafion® film. A number of studies have demonstrated that the perfluorinated ion-exchange polymer Nafion® is an excellent support for metal catalysts due to its high-developed surface area and conducting capacity [11, 22, 23]. Moreover, it has previously shown a good stability during electrolyses performed in a flow electrochemical cell, in aqueous medium [11]. Cyclic voltammetry was used to study the catalytic activity of immobilized titanocene and also to optimize the experimental conditions to prepare titanocene/Nafion®-modified electrodes.

3.1.1. Cyclic voltammetry analyses

The electrochemical behavior of the modified electrodes was investigated in acidic medium (0.5 M H_2SO_4) and hydroalcoholic medium (10:90 ethanol/0.5 M H_2SO_4). Spontaneous hydrolysis of titanocene dichloride in H_2SO_4 is known to give the oxidized form of the catalyst $(C_5H_5)_2Ti(OH_2)_2^{2+}$ [24, 25] that is stable in strong acidic medium, whereas it loses a cyclopentadienyl group at neutral pH [24]. Thus, the reversible system corresponding to $Ti^{IV/III}$ ($(C_5H_5)_2Ti(OH_2)_2^{2+}$ / $(C_5H_5)_2Ti(OH_2)_2^{+}$) (Eq. 1) was observed at -0.45 V/SCE by cyclic voltammetry (Fig. 2), confirming the presence of titanocene immobilized on the electrode.

$$(C_5H_5)_2Ti(OH_2)_2^{+} \rightleftharpoons (C_5H_5)_2Ti(OH_2)_2^{2+} + e^- \qquad \text{(Eq. 1)}$$

The electrochemical signal clearly depends on the solvent. Indeed, the voltammogram is well-defined in hydroalcoholic medium and exhibited a higher current. This result is in agreement with a previous work that has demonstrated that alcohols penetrate further into the perfluorinated regions than water [20, 22], which ensures therefore a good solvation of the active sites. From this observation, the hydroalcoholic medium was selected for further analyses.

3.1.2. Optimisation of the conditions to prepare modified electrodes

The preparation of the modified electrode was optimized varying the initial concentrations of Nafion® and titanocene and the sonication time with dipping of the graphite felt in the

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solution (Nafion®+ titanocene). Cyclic voltammetry analysis of the immobilized complex performed in hydroalcoholic medium was used to characterize the modified electrode. The integration of the surface area under the current-potential curve corresponding to the anodic peaks of the $Ti^{III/IV}$ system allows the evaluation of the number of moles of immobilized catalyst using the Faraday law:

$$\mathbf{Q = n_{e^-} NF = S/V}$$

With Q the electric charge (C), n_{e^-} the number of electron (1 e^-), N the number of moles of immobilized catalyst (mol), F the Faraday constant (F= 96500 C mol^{-1}), S the surface area of the current-potential curve and V the scan rate (V s^{-1}).

3.1.2.1. Effect of the concentration of Nafion®

To establish the influence of the concentration of Nafion® on the electrochemical signal of the immobilized catalyst, the modified electrodes were prepared by varying the concentration of Nafion® (5%) in ethanol from 0.4 to 1% keeping constant the initial concentration of titanocene to 2 g L^{-1} and the sonication time to 30 min.

The voltammograms of the modified electrodes performed in hydroalcoholic medium (10:90 Ethanol/H_2SO_4) are given in Fig. 3a. The increase of the concentration of Nafion® led to an increase of the electrochemical signal with a maximum at 0.8%. This behavior can be explained by a higher viscosity of the titanocene/Nafion® solution that leads to a better adhesion of the film on the graphite fibers. However, beyond 0.8% the concentration of Nafion® becomes limiting. This behavior is also well-underlined by the integration of the surface area of the current-potential curve, showing a decrease of the calculated number of moles of immobilized catalyst from 4.3 to 2.9 × 10^{-7} mol when the concentration of Nafion® increases from 0.8 to 1% (Fig. 3b). Such decrease can be due to the difficulty of the titanocene/Nafion® solution to go through the 3D electrode during its preparation and to an increase of the charge transfer barrier from the immobilized catalyst to the carbon surface.

Therefore, a suitable concentration of Nafion® of 0.8% was chosen for the preparation of the modified electrodes in order to increase the amount of immobilized catalyst while keeping a good charge transfer.

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3.1.2.2. Effect of the initial concentration of catalyst

Given that the modification of electrode is performed by adsorption of a Nafion® film containing the catalyst, the initial concentration of titanocene appears to be an important parameter which can influence the amount of titanocene incorporated into the Nafion® film. The initial concentration of titanocene has been optimized in the range of 0.5 to 4 g L^{-1} while keeping constant the concentration of Nafion® (0.8%) and the sonication time (30 min). The evolution of the number of moles of supported catalyst, estimated by cyclic voltammetry analysis, as a function of the initial catalyst concentration is shown in Fig. 4.

The initial concentration of titanocene increased with the number of moles of immobilized titanocene to reach a plateau for concentrations higher than 2 g L^{-1}. In addition, a significant uncertainty is noted for high concentrations, which seems to be due to the limited solubility of titanocene in ethanol. It is also important to mention that for initial concentrations of titanocene lower than 0.5 g L^{-1}, no electrochemical signal of the immobilized catalyst could be observed by cyclic voltammetry. To prepare modified electrodes with a high amount of catalyst and a good reproducibility, an initial concentration of titanocene of 2 g L^{-1} was therefore considered thereafter.

3.1.2.3. Effect of the sonication time

Once the concentrations of Nafion® (0.8%) and of catalyst (2 g L^{-1}) were optimized, the best sonication time, i.e the dipping time of the electrode in the Nafion® solution containing the catalyst, was determined.

The voltammograms recorded for a modified electrode prepared at different sonication times ranging from 15 to 45 min showed a slight decrease of the peaks when the sonication times increased (Fig. 5a).

This was confirmed by the integration of the current-potential curve corresponding to the anodic peaks, since the number of moles of titanocene immobilized on graphite felt shows a very slight decrease in function of time (Fig. 5b). A sonication time of 30 min was selected for the preparation of the modified electrode because a better reproducibility was obtained under these conditions.

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3.1.3. Characterization of the titanocene/Nafion®-modified electrode

The electrode modified considering the optimal conditions (initial concentration of titanocene 2 g L^{-1}, initial concentration of Nafion®: 0.8% and sonication time: 30 min) was analyzed by cyclic voltammetry in hydroalcoholic medium and by scanning electron microscopy coupled with an EDS detector (energy dispersive spectroscopy).

3.1.3.1. Cyclic voltammetry analysis

A typical voltammogram of the titanocene/Nafion®-modified graphite felt electrode prepared under the optimal conditions is given in Fig. 6.

The electrochemical signal evolved during successive scans and was stabilized after 5 scans (Fig. 6a). Since the catalyst is encapsulated in the Nafion® film, this phenomenon probably corresponds to an improvement of ionic conductivity inside the film during cycling.

The catalytic activity of the titanocene complex toward the reduction of metronidazole was then examined by cyclic voltammetry by addition of metronidazole (Fig. 6b). A considerable increase of the cathodic peak current corresponding to the reduction of Ti^{IV} in Ti^{III} was observed while the reverse anodic peak decreased, underlining the catalytic activity of immobilized titanocene. A small peak was noticed at -0.33 V/SCE and corresponded to the direct reduction of metronidazole at the electrode (Fig. 6b). The presence of this peak indicates a probable competition between the indirect and direct reduction of metronidazole at the electrode.

The amount of catalyst immobilized on the graphite felt electrode was roughly estimated to $4.32 \pm 0.06\ 10^{-7}$ mol based on 7 reproducibility measurements. As previously underlined in Fig. 4, the low uncertainty shows a good reproducibility of the method to prepare the modified electrode.

3.1.3.2. Analysis by Scanning Electron Microscopy equipped with an EDS detector (SEM-EDS)

A fresh graphite felt and a titanocene/Nafion®-modified electrode were analyzed by scanning electron microscopy (SEM) (Fig. 7).

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The comparison of the fresh graphite felt (Fig. 7a) with the modified graphite felt (Fig. 7d and 7f) clearly highlighted a coating by Nafion® of the fibers (in white) that are on the surface (Fig. 7d) and inside (Fig. 7f) the felt. A good porosity was maintained after modification.

As observed on Fig. 7b, c and e, some clusters of Nafion® were formed on the fibers. However, the images taken at higher magnification (× 5000) also revealed the presence of a thin Nafion® film covering the fibers.

EDS experiment was performed to confirm the presence of titanocene on the electrode surface (Fig. 8).

Spectrum 1 performed on the dark area of the fiber contains mainly carbon and oxygen atoms, showing that it corresponds to the carbon surface. The elements attesting the presence of titanocene (Ti, Cl) and Nafion® (F, S) were observed in spectra 2 and 3, whereas the quantification of minor elements on heterogeneous samples was difficult by EDS [26]. These results clearly highlight the presence of titanocene in the Nafion® film.

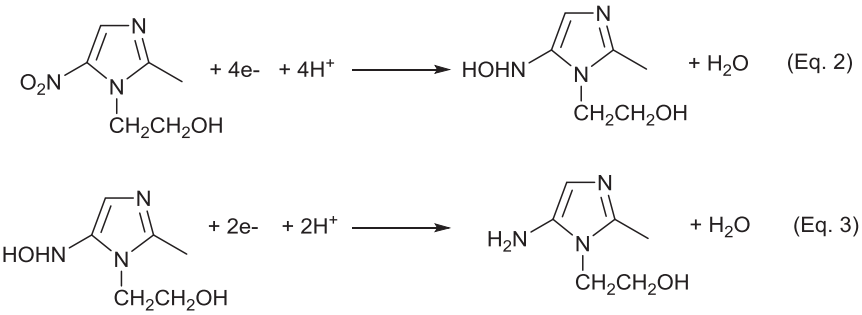
3.2. Application of titanocene/Nafion®-modified electrode for electrocatalytic reduction of metronidazole

3.2.1. Degradation of metronidazole

The electrodes modified in the optimum conditions (initial concentration of titanocene 2 g L^{-1}, initial concentration of Nafion®: 0.8% and sonication time: 30 min) were used as working electrodes for the reduction of metronidazole (C = 100 mg L^{-1}, V = 100 mL) in a flow electrochemical cell. The reduction of metronidazole was first carried out on graphite felt modified by a Nafion® film without catalyst to check the effect of the catalyst on the reduction of metronidazole.

Two reaction processes can occur during the electrolysis. The first one is the direct electrochemical reduction of metronidazole on the graphite felt electrode involving a four-electron reduction of the nitro group into hydroxylamine (Eq. 2) and a subsequent two-electron reduction into the amino derivative (Eq. 3) [18].

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The second one is the catalytic reduction of metronidazole into its amino derivative (Eq. 4). It is worth noting that this six-electron reduction does not give rise to the formation of a hydroxylamine intermediate as for direct cathodic reduction.

$$6(C_5H_5)_2Ti(OH_2)_2^{+} + RNO_2 + 7H^{+} \longrightarrow 6(C_5H_5)_2Ti(OH_2)_2^{2+} + RNH_3^{+} + 2H_2O \quad \text{(Eq. 4)}$$

The evolution of the conversion yield showed that cathodic reduction of metronidazole occurred with and without catalyst, underlining that both direct and indirect reduction processes took place (Fig. 9). However, the efficiency was higher when the catalyst was immobilized on the electrode and conversion yields of 99.9% were reached after 60 min of electrolysis. Moreover, results were similar in aqueous and hydroalcoholic medium. Even if the hydroalcoholic medium gives a higher signal in cyclic voltammetry, it does not seem to affect the performances of the electrolysis. All electrolyses were therefore performed in aqueous acidic medium thereafter.

A comparison between heterogeneous catalysis performed on a titanocene/Nafion®- modified electrode and homogeneous catalysis was done. To obtain a reduction efficiency similar to heterogeneous catalysis and a conversion yield close to 100% after 60 min (Fig. 10), a substrate/catalyst ratio of 15 was necessary in homogeneous catalysis.

In heterogeneous catalysis, if all metronidazole in solution is considered, the substrate/catalyst ratio is 136, underlining the interest of heterogeneous catalysis that requires less catalyst. Advantageously, in the 3D electrode, where the reaction takes place, a small amount of the substrate is actually forced into intimate contact with an “excess” of catalyst. Thus, if only the volume of solution inside the graphite felt is considered to calculate the substrate/catalyst ratio, a value of 1.2 is obtained (Table 1). This high substrate/catalyst ratio explains the high reaction efficiency obtained in heterogeneous catalysis.

Current efficiencies were around 70% for both processes. These values are good since a competition with water reduction already occurs on graphite felt at the electrolysis potential (-

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0.5 V/SCE) in strong acidic medium. Heterogeneous catalysis also led to an improvement of the turnover number from 14.5 to 139 compared with homogeneous catalysis. As it has already been observed in supported catalysis [27-29], the catalyst is more stable when it is immobilized on the electrode.

3.2.2. Evolution of the biodegradability after electroreduction on modified electrode

In order to assess the effect of supported catalysis on biodegradability, the biological oxygen demand (BOD_5) and chemical oxygen demand (COD) values were measured after 60 min of electrolysis on the modified electrode and the BOD_5/COD ratio was used as a biodegradability indicator [30, 31].

As highlighted in table 2, the BOD_5/COD ratio significantly increased from 0.06 before electrolysis to 0.35 after electrolysis. This result indicates the efficiency of the electrocatalytic reduction on titanocene/Nafion® modified-electrode to change metronidazole in more biodegradable by-products.

If compared with homogeneous catalysis, the catalyst immobilization slightly improved the biodegradability of the solution after electrolysis. Indeed, the presence of the catalyst in solution can have an impact on biodegradability as previously suggested [18], and underlines the advantage of heterogeneous catalysis.

3.2.3. Stability of the titanocene/Nafion®-modified electrode

The titanocene/Nafion®-modified electrode was analyzed after 60 min of electrolysis by SEM-EDS and by cyclic voltammetry to check its stability.

SEM analysis showed the presence of Nafion® inside and on the graphite felt surface (Fig.11a,b), indicating the mechanical stability of the Nafion® film during the electrolysis in the flow cell. In addition, EDS analysis (Fig. 11c) revealed the presence of the elements constitutive of Nafion® (F, S) and the titanium catalyst (Ti), showing that it is still present inside the Nafion® film after electrolysis. It is worth noting that chlorine atom has not been detected by EDS analysis, that is consistent with a total hydrolysis of titanocene dichloride into the $(C_5H_5)_2Ti(OH_2)_2^{2+}$ complex.

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However, cyclic voltammetry analyses of the titanocene-Nafion® modified electrode after electrolysis (60 min) showed a decrease of approximately 65% of the electrochemical signal of the $Ti^{IV/III}$ system (Fig. 12), which corresponds to a decrease of the amount of immobilized catalyst from 4.32 10^{-7} mol before electrolysis to 1.5 10^{-7} mol after electrolysis. This loss of electrochemical activity of the electrode could be due to a slow leaching of the catalyst in solution or to its deactivation.

This phenomenon has already been described in literature. Indeed, cationic metal-complex catalysts successfully encapsulated in a Nafion® film has already shown leaching occurring during catalysis and in several instances the supported catalyst has been reused with a little loss of activity [22]. To check the effect of the loss of activity observed by cyclic voltammetry on the effectiveness of the modified electrode, 3 successive electrolyses of 60 min were performed on the same modified graphite felt electrode. Fig. 13 shows the evolution of the metronidazole conversion yield with time for the 3 successive electrolyses.

A slight decrease in the efficiency of metronidazole reduction was observed after each electrolysis of 60 min, showing a slight loss of catalytic activity. However, the evolution of the conversion yield with time after three electrolyses with the titanocene/Nafion®-modified electrode remains still faster than with the graphite felt modified by a Nafion® film without catalyst (blank). This result highlighted the efficiency and the good stability of the modified electrode after 3 successive electrolyses. The decrease of 65% of the electrochemical signal of the catalyst observed by cyclic voltammetry and probably due to a loss of catalyst in solution does not seem to strongly affect the effectiveness of metronidazole reduction, showing that the catalyst is efficient even in lower concentration.

3.2.4. By-products analysis

After reduction of metronidazole on the titanocene/Nafion®-modified electrode (100 mg L^{-1}, V= 100 mL, 0.5M H_2SO_4, E= -0.5 V/SCE, t= 60 min), the solution was analyzed by UPLC-MS/MS.

UPLC-MS/MS analyses of the electrolyzed solution revealed the presence of the expected amino derivative **1** (Scheme 1), as well as azo and azoxy compounds **2** and **3**, as previously observed in homogeneous catalysis [32] (see supplementary material).

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Since the formation of the dimers **2** and **3** is due to the presence of hydroxylamine as an intermediate [18], it confirms that direct reduction of metronidazole occurred on the graphite felt electrode, due to a deficiency of titanocene on some areas of the graphite fibers. However, the analysis by LC-MS/MS did not allow to quantify the identified by-products and to really compare the selectivity of heterogeneous and homogeneous catalyses.

4. Conclusions

In this work, the electrocatalytic reduction of metronidazole using a catalyst immobilized on a graphite felt electrode was studied. A titanium complex was immobilized onto a graphite felt in a Nafion® film. The preparation of the modified electrode was optimized varying the initial concentrations of Nafion® and titanocene, and the sonication times. The amount of titanocene immobilized on the graphite felt in optimized conditions was estimated to $4.32 \pm 0.06\ 10^{-7}$ mol/cm^3 by cyclic voltammetry. In addition, a good distribution of the Nafion® film inside and on the electrode surface was shown by SEM analysis. However, the fibers were not totally covered by the Nafion®/titanocene film, as observed by EDS analysis. The catalytic performances of the titanocene/Nafion®-modified electrodes for the reduction of metronidazole underlined the efficiency of the process: a total reduction was obtained after 60 min of electrolysis on modified electrode with the formation of more biodegradable by-products than metronidazole. Comparison of homogeneous and heterogeneous catalyses highlighted a higher stability of the catalyst, giving rise to a higher turnover number, and an improvement of biodegradability. The stability of the modified electrode was studied after electrolysis. The SEM-EDS analysis show a good stability of the titanocene/Nafion® film after electrolysis. Cyclic voltammetry analysis suggests a decrease in catalytic activity, which may be due to the leaching of the titanocene in solution or to its deactivation. However, this loss of activity does not appear to significantly affect the efficiency of the modified electrode after 3 successive electrolyses.

Supporting information

Chromatograms and mass spectra of metronidazole and by-products.

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Fig. 1: Preparation of the modified electrodes.

Fig. 2: Typical voltammogram of titanocene/Nafion®-modified electrode recorded at 20 mV s^{-1} in 0.5 M H_2SO_4 (- - -) and in hydroalcoholic medium 90% H_2SO_4 + 10% EtOH (——). Experimental conditions of the preparation of the modified electrode: $[(C_5H_5)_2TiCl_2]_{initial}$ =2 g L^{-1}, [Nafion®] = 0.8%, $t_{sonication}$= 30 min. The 10th scan is given.

Fig. 3: Cyclic voltammogram of the modified electrodes (a) and evolution of the calculated number of moles of immobilized titanocene (b) prepared at different concentrations of Nafion® (from 0.4 to 1% in ethanol) for a given concentration of titanocene (2 g L^{-1}) and a sonication time of 30 min. Voltammograms were recorded at 20 mV s^{-1} in hydroalcoholic medium (90% H_2SO_4 + 10% EtOH). Error bars are based on 2 to 7 reproducibility measurements. The 10th scan is given.

Fig. 4: Evolution of the number of moles of catalyst immobilized on the graphite felt electrode, estimated by cyclic voltammetry in hydroalcoholic medium (90% H_2SO_4 + 10% EtOH), as a function of the initial catalyst concentration. Experimental conditions of the preparation of modified electrodes: $[(C_5H_5)_2TiCl_2]_{initial}$ = 0.5 - 4 g L^{-1}, $[Nafion®]_{initial}$ = 0.8%, $t_{sonication}$= 30 min. Error bars are based on 2 reproducibility measurements.

Fig. 5: a) Cyclic Voltammogram of modified electrodes prepared using different sonication times (from 15 min to 45 min) Error bars are based on 2 reproducibility measurements and b) evolution of the number of moles of immobilized catalyst on graphite electrode for given amounts of titanocene (2 g L^{-1}) and Nafion® (0.8%) Voltammograms were recorded at 20 mV s^{-1} in hydroalcoholic medium (90% H_2SO_4 + 10% EtOH). The 10th scan is given.

Fig. 6: a) Typical voltammogram in hydroalcoholic solution (90% H_2SO_4 + 10% EtOH) of the electrode modified using the optimal conditions ($[Nafion®]_{initial}$ = 0.8% , $[(C_5H_5)_2TiCl_2]_{initial}$ =2 g L^{-1} and $t_{sonication}$ = 30 min) during 20 scans and b) after 6 scans (—) in the presence of metronidazole (100 mg/L) (------). Voltammogram of metronidazole (100 mg/L) (…….) was recorded at a Nafion modified electrode ($[Nafion®]_{initial}$ = 0.8% and $t_{sonication}$ = 30 min) for comparison. Scan rate : 20 mV s^{-1}

Fig. 7: SEM images of a fresh graphite felt (× 5000) (a) and of graphite felt modified in the optimum conditions (image of fibers on the electrode surface (Magnification ×1500 (b), × 3000 (c), × 5000 (d)) and inside the electrode (Magnification × 3000 (e), × 5000 (f)).

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Fig. 8: Chemical composition measured by EDS analysis of the graphite felt electrode modified using the optimum conditions at 3 different positions.

Fig. 9: Conversion yield of MNZ solution *versus* electrolysis time performed on Nafion®- (- - ■ - -) and titanocene/Nafion®-modified graphite felt (—●—) in aqueous acidic medium (0.5M H_2SO_4). The same experiment was carried out with the titanocene/Nafion®-modified electrode in hydroalcoholic medium (—▲—). Electrolysis conditions: E=-0.5 V/SCE, [MNZ] =100 mg L^{-1}, V = 100 mL. Error bars are based on 2 reproducibility measurements

Fig. 10: Conversion yield of metronidazole *versus* time after electrolysis on a graphite felt electrode in homogeneous catalysis (10 mg L^{-1} of titanocene) (—■—) and heterogeneous catalysis (···●···). Electrolyses conditions: E=-0.5 V/SCE, [MNZ] =100 mg L^{-1}, V = 100 mL, 0.5 M H_2SO_4, t=60 min. Error bars are based on 2 reproducibility measurements.

Fig. 11: SEM image inside the electrode (Magnification × 1000) (a), on the electrode surface (Magnification ×1000) (b) and EDS results (c) of the titanocene/Nafion®-modified graphite felt electrode (black square) after electrolysis of metronidazole in the flow cell. Electrolysis conditions: [MNZ] = 100 mg L^{-1}, V= 100 mL, 0.5M H_2SO_4, E= -0.5 V/SCE, t= 60 min.

Fig.12: Cyclic voltammetry of a titanocene-Nafion® modified electrode before (- - - - -) and after electrolysis (——). Voltammograms were recorded in hydroalcoholic medium *(*90% H_2SO_4 + 10% EtOH) at 20 mV s^{-1}.

Fig.13: Evolution of the conversion yield of metronidazole with time after 3 successive electrolyses carried out on the same modified graphite felt electrode. Error bars are based on 2 reproducibility measurements.

Scheme and figure captions:

Scheme 1: chemical structure of the products resulting from the reduction of metronidazole.

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Figure 1

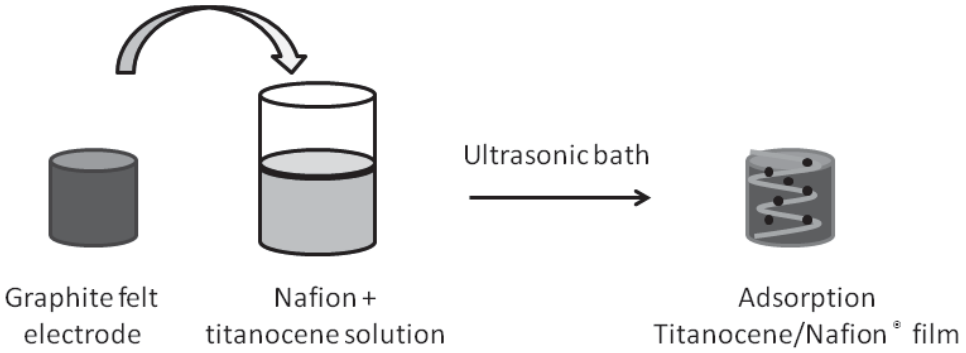
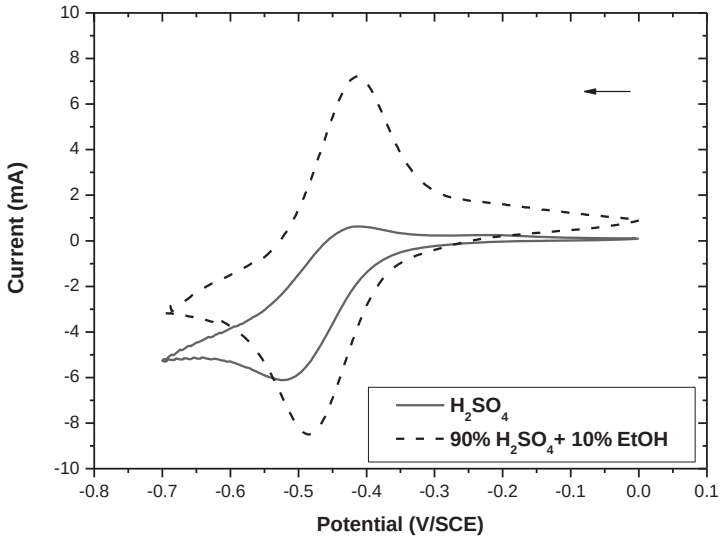
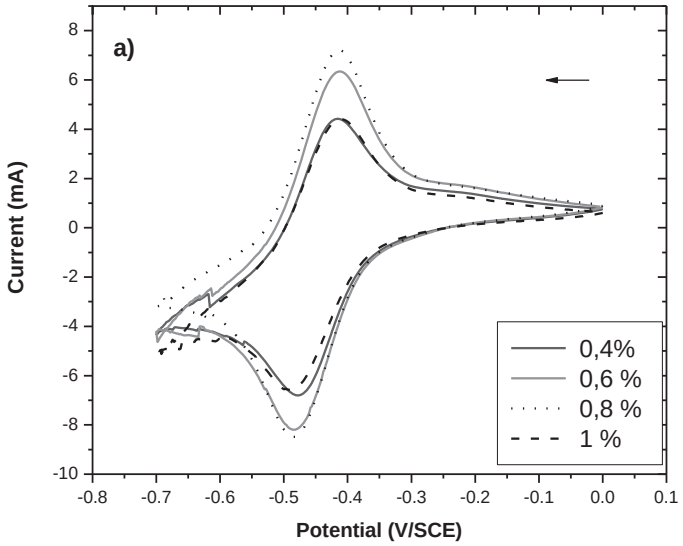


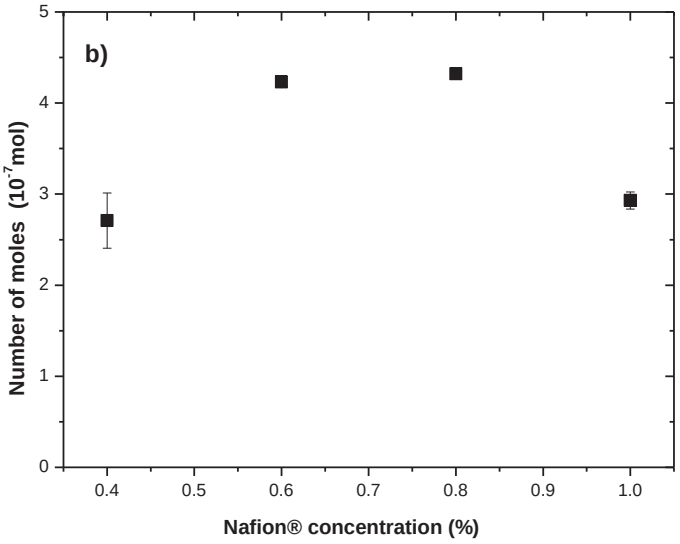
Figure 2



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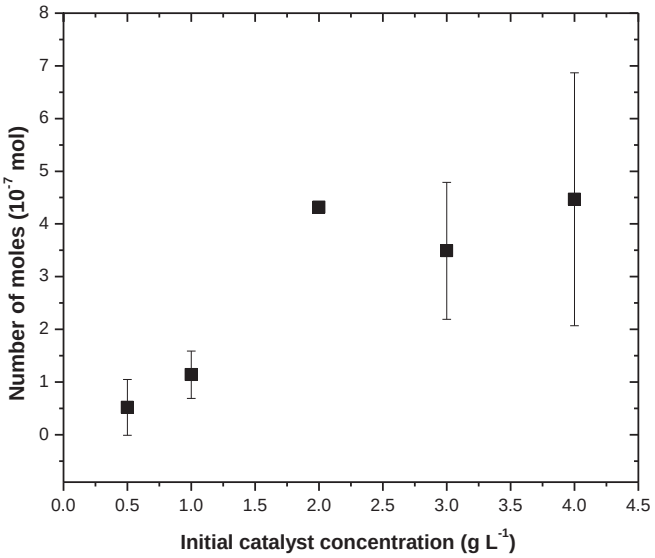
Figure 3





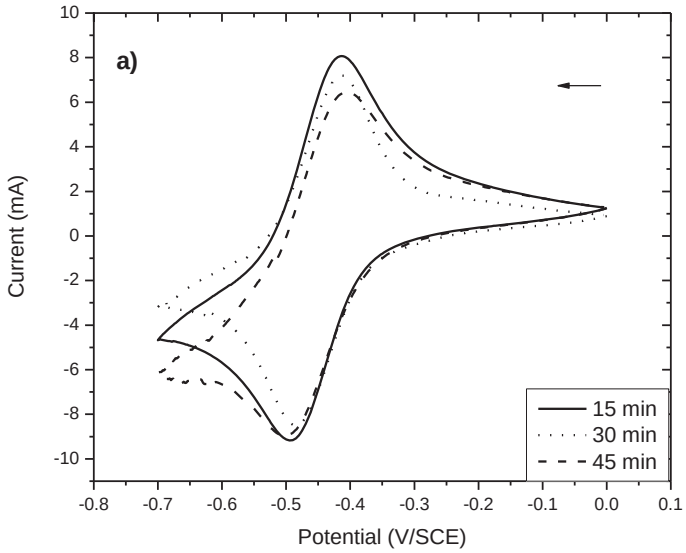
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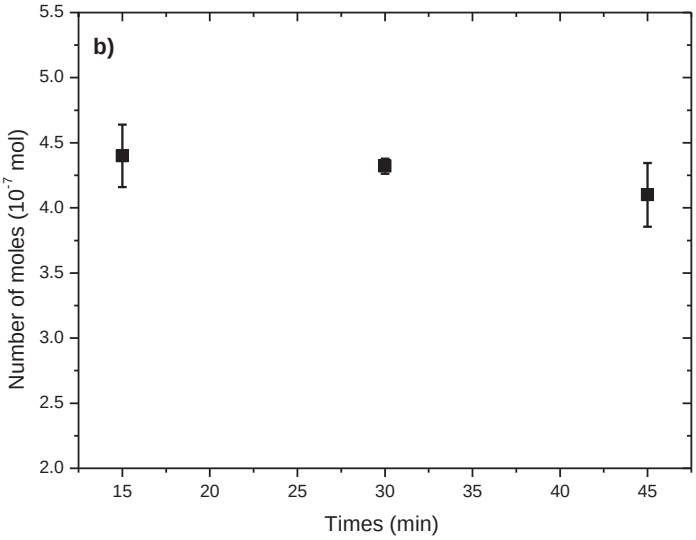
Figure 4



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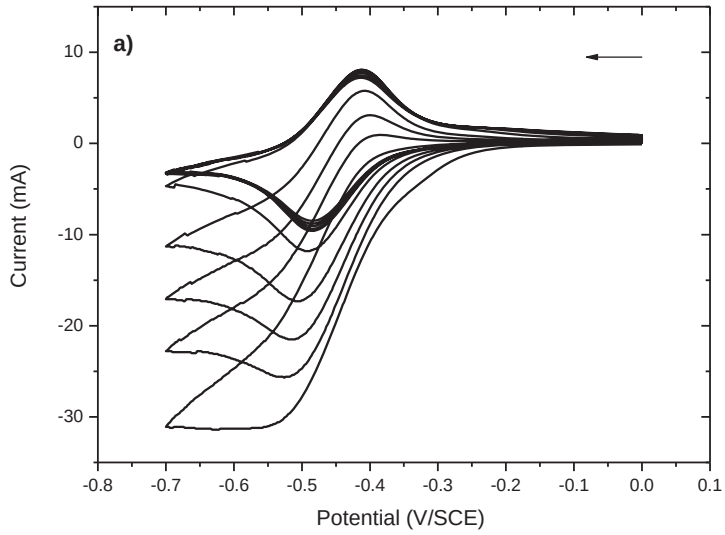
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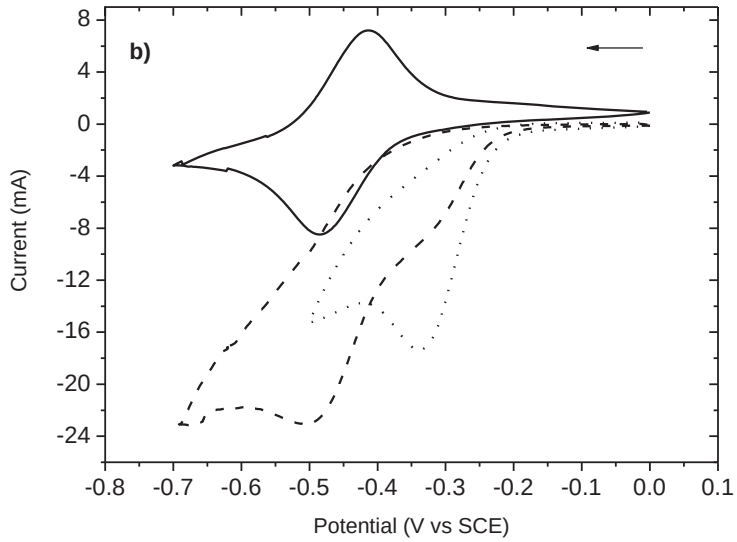




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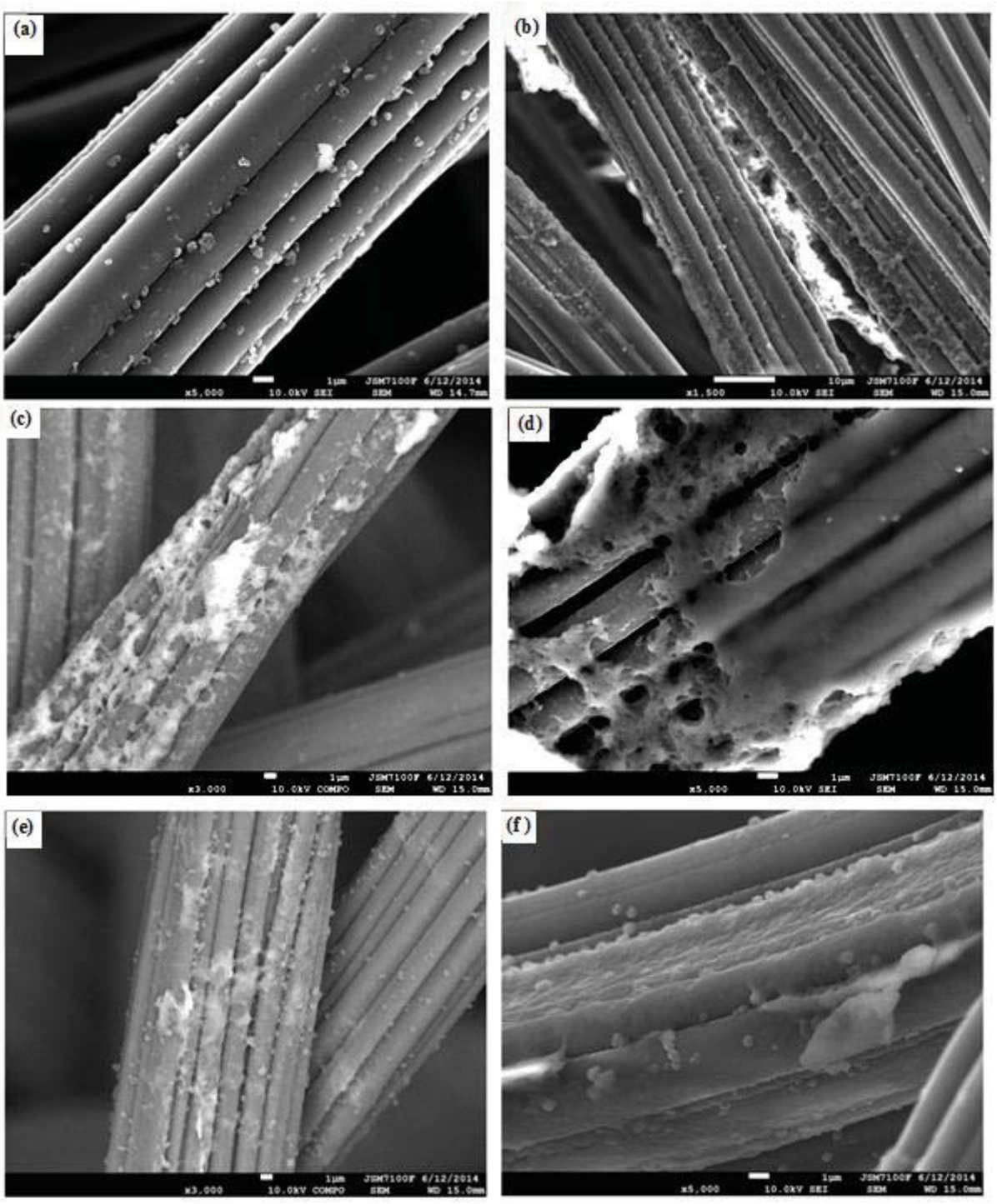
Figure 6





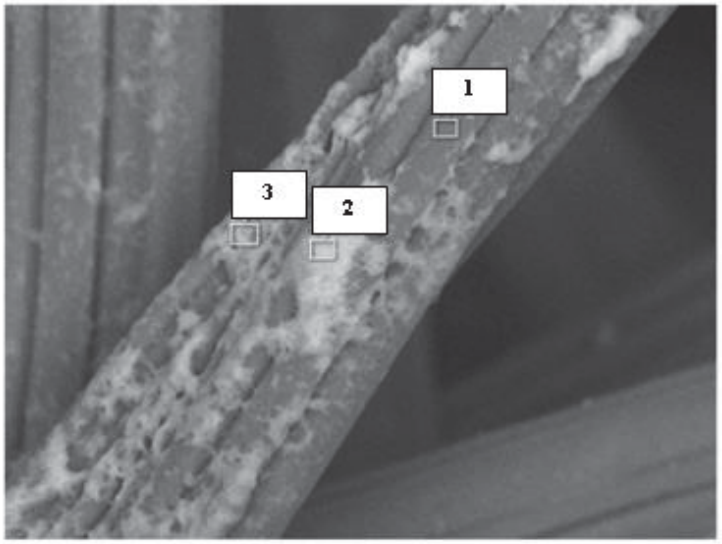
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Figure 7



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Figure 8



Element	% atomic		
	Spectrum 1	Spectrum 2	Spectrum 3
C	27.2	26.9	30.7
O	72.4	56.9	62.2
F	0.3	13.7	6.7
S	-	0.2	0.1
Cl	-	1.1	0.1
Ti	-	1.2	0.3

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Figure 9

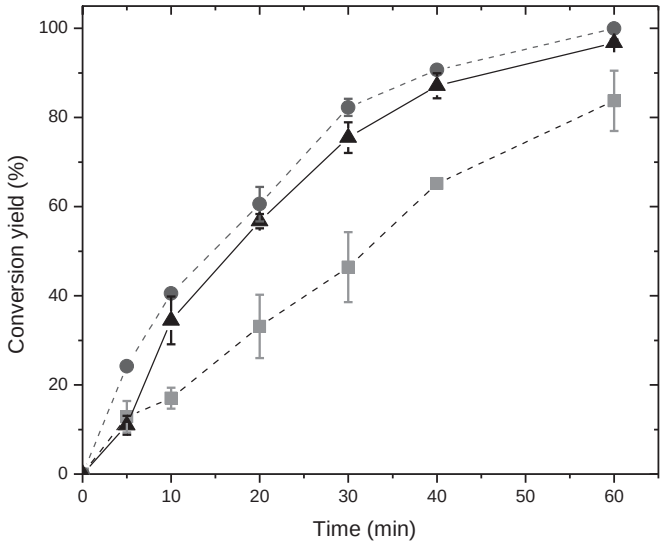
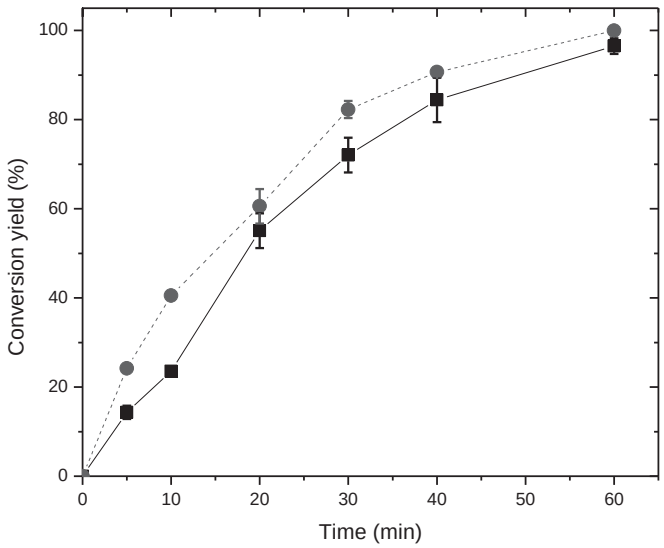
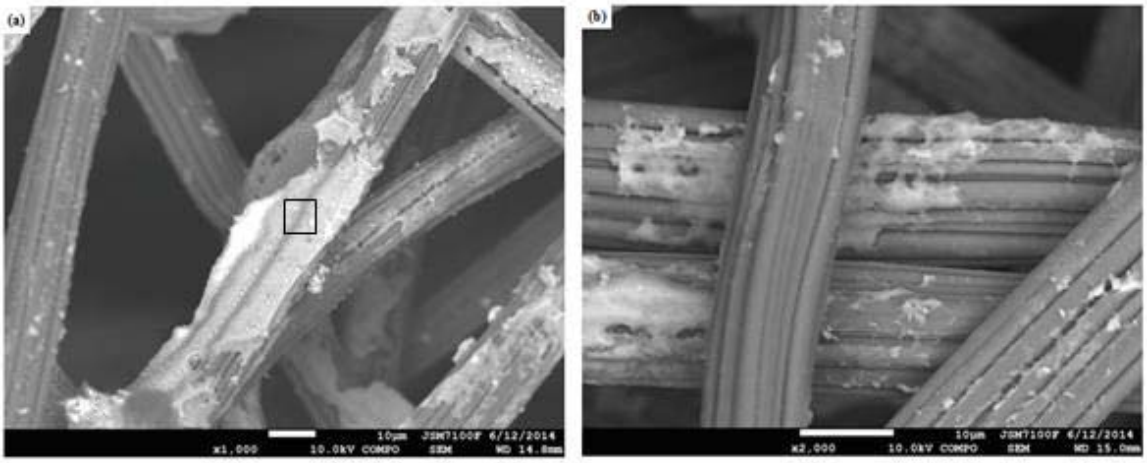


Figure 10



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Figure 11



Element	% atomic
C	54.51
O	2.99
F	40.30
S	1.07
Ti	1.13

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Figure 12

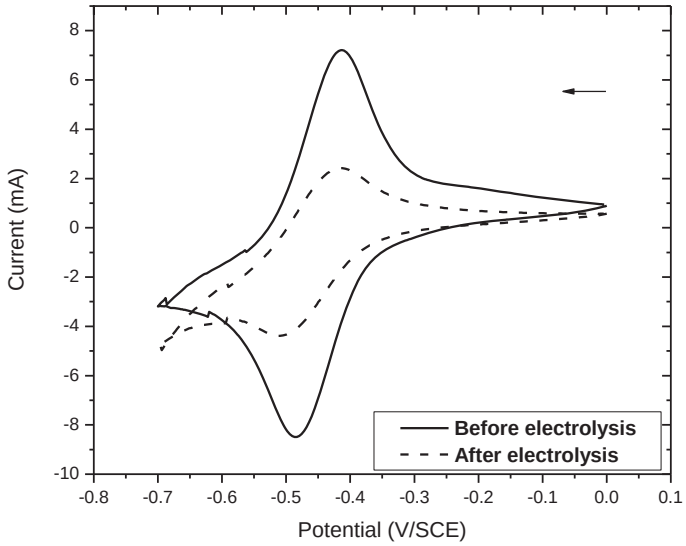
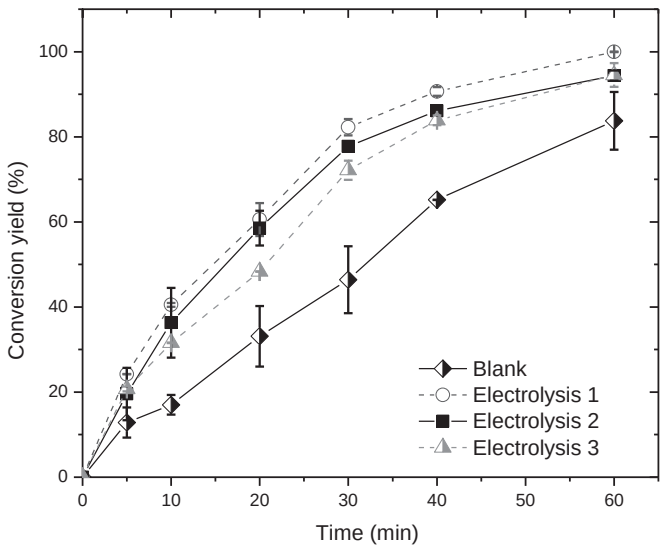
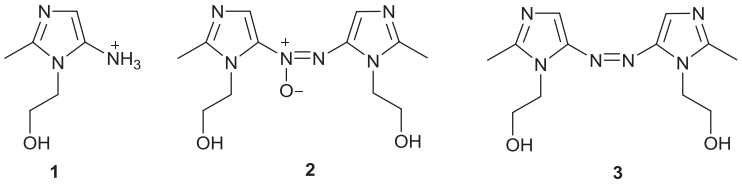


Figure 13



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Scheme 1



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Table 1: Cathodic reduction of metronidazole ([100 mg L^{-1}], V = 100 mL, 0.5 M H_2SO_4) in homogeneous and heterogeneous catalysis.

	Homogeneous catalysis	Heterogeneous catalysis
Catalyst[a] (mol)	4×10^{-6}	$4.3 \times 10^{-7} \pm 5.9 \times 10^{-9}$
Conversion yields[b] (%)	96.5 ± 1.7	100 ± 0
Substrate/catalyst ratio	15	1.2
Turnover number	14.5	139
Current efficiency[c] (%)	71	70

[a] Based on 7 reproducibility measurements, [b] Calculated using the concentrations determined by HPLC analyses, Conversion yields = ($n_{metronidazole} \times 100$) / $n_{metronidazole\ initial}$ with $n_{metronidazole}$ and $n_{metronidazole\ initial}$ the number of moles of metronidazole at the end and at the beginning of the electrolysis, respectively. Based on 2 reproducibility experiments, [c] Current efficiency = 100×theoritical charge / charge passed

Table 2: Biodegradability of metronidazole and after electrolysis in the presence of the catalyst in solution and immobilized on the electrode. Electrolysis conditions: [MNZ] = 100 mg L^{-1}, V = 100 mL, 0.5 M H_2SO_4, E= -0.5 V/SCE, t= 60 min.

	Solution of metronidazole	Homogeneous catalysis	Heterogeneous catalysis
COD[a]	81	98.5 ± 0.5	92.5 ± 3.5
BOD_5[a]	5	29	32 ± 5.7
BOD_5 /COD	0.06	0.29 ± 0.1	0.35 ± 0.05

[a] based on 2 reproducibility experiments