Carbon Nanotubes Modified with SnO₂ Rods for Levofloxacin Detection

Vivian Cesarino, a Ivana Cesarino, *,b Fernando C. Moraes, b Sergio A. S. Machado b and Lucia H. Mascaro c

a Escola de Engenharia de São Carlos and b Instituto de Química de São Carlos, Universidade de São Paulo, CP 780, 13560-970 São Carlos-SP, Brazil

b Departamento de Química, Universidade Federal de São Carlos, CP 676, 13565-905 São Carlos-SP, Brazil

department of Chemistry, Universidade Federal de São Carlos, CP 676, 13565-905 São Carlos-SP, Brazil

Um novo sensor baseado em nanotubos de carbono de paredes múltiplas modificado com bastões de SnO₂ foi desenvolvido para a determinação eletroquímica de levofloxacino. A morfologia, a estrutura, e o comportamento eletroquímico do eletrodo compósito foram caracterizados por microscopia eletrônica de varredura, energia dispersiva de raios X e voltametria cíclica, respectivamente. Voltametria de pulso diferencial em solução tampão fosfato pH 6,0 permitiu a aplicação de um método para determinar níveis de levofloxacino em um intervalo de 1,0-9,9 µmol L⁻¹, com limite de detecção calculado em 0,2 µmol L⁻¹ (72,0 mg L⁻¹).

A new sensor based on multi-walled carbon nanotubes modified with SnO₂ rods for the electrochemical determination of levofloxacin has been investigated. The morphology, the structure, and the electrochemical performance of the composite electrode were characterised by scanning electron microscopy, energy dispersive X-ray spectroscopy, and cyclic voltammetry, respectively. Differential pulse voltammetry in phosphate buffer solution at pH 6.0, allowed the application of a method to determine levofloxacin levels in a range of 1.0-9.9 µmol L⁻¹, with a limit of detection calculated at 0.2 µmol L⁻¹ (72.0 mg L⁻¹).

Keywords: carbon nanotubes, SnO₂ rods, levofloxacin

Introduction

One of the most widely used antibiotics in the world is levofloxacin, which is the third generation of fluoroquinolone antibiotics. In humans, after levofloxacin oral administration, approximately 87% of the dose may be recovered as unchanged structural drug in the urine. Consequently, the antibiotic is discarded in sewage, causing hazardous effects to human health and in the quality of life. Thus, studies on forms of wastewater treatment for the removal of these xenobiotic, as well as studies focused on the determination of these antibiotics to be carried out a monitoring of the wastewater are very important.

The development of versatile materials to modify electrodes has been the goal of some environmental analyses that use electrochemical methods to measure micropollutants. Nanostructured carbon materials, in particular the carbon nanotubes (CNTs), appear to be one of the most promising supporting materials for surface modification of electrodes, due to their unique properties. Their main properties include: high area/volume ratio, thermal and chemical stability, conducting or semiconducting behaviour, high surface area and the presence of functional groups anchored on the CNT edge making these materials an excellent support to be modified with several species.

Tin dioxide (SnO₂) is a material with versatile applicability in a large number of physicochemical procedures. This inorganic material has been extensively used in the photoelectronics, microelectronics, solar cells, sensing and biosensing devices, due to its relatively higher conductivity than TiO₂ and SiO₂. Due to the conductive properties, SnO₂ has been used for the modification of electrochemical transducers in sensor applications. Therefore, tin oxide displayed an interesting material to be used as a modifier of CNTs. In this study, the carbon nanotubes were modified with SnO₂ rods in the first time for antibiotic detection.

The usual analytical methods for levofloxacin determination are based on chromatographic methods such...
as liquid chromatography–mass spectrometry (LC-MS)\textsuperscript{14} and high performance liquid chromatography (HPLC),\textsuperscript{15} electrophoresis\textsuperscript{16} and UV-Vis.\textsuperscript{17} There are few reports available on the electrochemical detection of levofloxacin. In this case, these methods are based on the irreversible oxidation of the piperazine group of the levofloxacin moiety.\textsuperscript{18,19}

Considering all descriptions above, this study focused on the synthesis, characterisation and application of a composite based on multi-walled carbon nanotubes and SnO\textsubscript{2} rods (MWCNT-SnO\textsubscript{2}) for electrochemical determination of levofloxacin. The proposed composite could be an alternative material for determination of the antibiotic in environmental samples.

**Experimental**

**Apparatus and procedures**

Cyclic voltammetry (CV) and differential pulse voltammetry (DPV) experiments were performed using a model PGSTAT 128N Autolab electrochemical system (Eco Chemie, Netherlands) coupled to a computer and monitored with NOVA software. The electrochemical cell was assembled with a conventional three-electrode system: bare glassy carbon electrode (GC) and GC electrode modified with the hybrid multi-walled carbon nanotubes and SnO\textsubscript{2} rods (GC/MWCNT-SnO\textsubscript{2}) as working electrodes (3 mm diameter), an Ag/AgCl electrode in KCl (3.0 mol L\textsuperscript{−1}) as a reference electrode, and Pt wire as an auxiliary electrode. All experiments were carried out at a controlled temperature (25 °C). Electrochemical characterisation of the MWCNT-SnO\textsubscript{2} composite was performed using CV in 0.1 mol L\textsuperscript{−1} HCl with a scan rate of 50 mV s\textsuperscript{−1}. DPV measurements were obtained with a scan rate of 10 mV s\textsuperscript{−1}, pulse amplitude of 100 mV and a step potential of 2 mV, in a 0.1 mol L\textsuperscript{−1} phosphate-buffer solution (PBS) at pH 6.0 containing 100.0 µmol L\textsuperscript{−1} of levofloxacin.

The structure and morphology of the MWCNT-SnO\textsubscript{2} composite was characterised using a scanning electron microscope coupled to an energy-dispersive X-ray spectroscopy (SEM-EDX) and the images were recorded with a LEO-440 (Zeiss-Leica) microscope.

**Chemicals and solutions**

All solutions were prepared with water purified from a Barnested Nanopure System (resistivity ≥ 18 M\textOmega\textperiodcentered cm). All chemicals were of analytical grade and were used without further purification. Levofloxacin, tin chloride (SnCl\textsubscript{2}), and MWCNT (90\% purity) were obtained from Sigma-Aldrich (Germany).

**Synthesis of the MWCNT-SnO\textsubscript{2} rods composite**

Before the synthesis of the MWCNT-SnO\textsubscript{2} rods composite, the MWCNT was functionalised. For this, an amount of approximately 1.0 g of MWCNT was mixed with 500 mL of a 1:3 mixture of HNO\textsubscript{3}/H\textsubscript{2}SO\textsubscript{4} for 12 h. This was then filtered through a 0.45 µm Millipore Nylon\textsuperscript{®} filter membrane. The resulting MWCNT was continuously washed using distilled water until the pH of the filtrate was neutral, and then dried overnight in a vacuum oven at 120 °C. After, the synthesis of the MWCNT-SnO\textsubscript{2} rods composite was performed using sodium dodecyl sulfate (SDS) as a surfactant. A suspension containing a ratio in weight of 10:4 (MWCNT/SDS) was prepared in 20 mL of ethanol pure grade and sonicated for 20 min. An excess of sodium borohydride (80 mg of NaBH\textsubscript{4}) was added to this suspension and sonicated for a further 20 min. A solution containing 32 mg of SnCl\textsubscript{2}, which corresponds to 20\% (m/m) of Sn, was slowly dropped onto the MWCNT suspension, which was kept under constant stirring. Once the reaction was complete, the MWCNT-SnO\textsubscript{2} rods composite was dispersed using an ultrasonic probe for 1 h. Finally, the MWCNT-SnO\textsubscript{2} rods was filtered through a 0.45 µm Millipore Nylon\textsuperscript{®} filter membrane and washed with ultrapure water. The formed hybrid was then dried in a vacuum oven for 12 h at 60 °C.

**Preparation of the electrodes**

Prior to modification, the GC electrode surface was polished with 0.3 µm alumina slurries, rinsed thoroughly with double-distilled water, sonicated for 5 min in ethanol and 5 min in water, and dried in air. Two milligrams of the MWCNT-SnO\textsubscript{2} composite was suspended in 1.0 mL of ethanol containing 0.5% of Nafton\textsuperscript{®}.\textsuperscript{8} The suspension was dispersed using ultrasonic stirring for 20 min. A 15 µL aliquot of this dispersion was dropped onto the GC electrode surface, and dried at room temperature. For comparison, a GC electrode was modified with a film of SnO\textsubscript{2} electrodeposited in bulk condition. For this, the GC electrode was immersed in a 0.1 mol L\textsuperscript{−1} of HCl containing 0.1 mmol L\textsuperscript{−1} of SnCl\textsubscript{2}, and it was applied a work potential at −1.0 V during 120 s.

**Results and Discussion**

**Structural and morphological characterisation of the MWCNT-SnO\textsubscript{2} composite**

The structural characterisation of the MWCNT-SnO\textsubscript{2} composite was evaluated using the EDX patterns, as shown...
in Figure 1. The EDX patterns of the MWCNT in absence of SnO$_2$ rods (Figure 1A, inset) presented a typical carbon reflection peak at 0.3 keV, which can be attributed to the graphite of the carbon nanotubes. The weight percentage of carbon was calculated at 89.4%. Oxygen atoms were detected at 0.6 keV with weight percentage calculated at 10.6%. The presence of oxygen in the CNT sample is due to air oxidation and the previous functionalisation procedure, in which hydroxyl and carboxyl groups were anchored onto the CNT surfaces. Figure 1A displays typical images of MWCNT. The MWCNT have an average length of 1.0 µm and the diameter estimated at 50 to 80 nm.

Figure 1A displays the EDX patterns of the MWCNT-SnO$_2$ composite. The peaks corresponding to the carbon and oxygen appear at 0.3 and 0.5 keV, respectively. However, after the composite synthesis, the EDX patterns presented a series of peaks between 3.4 and 4.2 keV, which were attributed to the presence of Sn. The composite composition was calculated from EDX spectra at different places of the samples, and the following average weight percentages were observed: 36.9% of carbon, 43.6% of tin and 19.4% of oxygen. The results showed that the oxygen percentage increases when MWCNT was modified with tin, what indicates that the tin species deposited was SnO$_2$. The SEM image of the composite materials is presented in the inset of Figure 1B, where it is clearly possible to see the formation of SnO$_2$ rods (shown by arrows) dispersed throughout the MWCNT, with average length estimated in the range between 1-3 µm and the rod diameter varying in the range between 2 to 5 µm.

**Electrochemical characterisation of the MWCNT-SnO$_2$ composite**

The presence of Sn on the composite electrode was characterised electrochemically. As a result, the CV experiments were carried out in 0.1 mol L$^{-1}$ of HCl, with a scan rate of 50 mV s$^{-1}$. The electrochemical behaviour of the MWCNT-SnO$_2$ composite was compared with the voltammetric response of a glassy carbon electrode modified with a SnO$_2$-electrodeposited film. In the cyclic voltammograms shown in Figure 2, both the GC electrode modified with Sn electrodeposited film (curve a) and the GC electrode modified with MWCNT-SnO$_2$ composite (curve b) showed a quite similar reduction and oxidation processes.

In curve a, at a potential value of about −0.64 V the reduction of Sn(IV) to Sn (0) is observed and the oxidation to Sn(II) occurred at −0.41 V. In accordance with literature, oxidation to Sn(II) and Sn(IV) occurs at more positive potential, at 1.0 V.$^{21,22}$ The same behaviour was observed in curve b, the reduction process appeared at −0.65 V and the oxidation in −0.46 V. The slight differences in the peak potential of the oxidation and reduction of tin and the fact that the peaks are narrower in curve b are characteristic of the presence of the CNTs and SnO$_2$ microparticles in rod-like form. For curve a, the electrode is a continuous film of the SnO$_2$. In this case, the presence of CNTs and rods promotes an increase in the Sn redox process. However, the electrochemical process...
observed in the composite electrode demonstrated that the CNTs are modified with Sn.

**Electrochemical studies of levofloxacin**

The electrochemical behavior of levofloxacin at the GC/MWCNT-SnO\(_2\) electrode was evaluated using DPV measurements. The DPV voltammograms were collected in 0.1 mol L\(^{-1}\) PBS at pH 6.0 containing 100 µmol L\(^{-1}\), using a scan rate of 10 mV s\(^{-1}\), pulse amplitude of 100 mV and a step potential of 2 mV, and the results obtained are presented in Figure 3. In the absence of levofloxacin (dotted line), no electrochemical process was observed in the potential range studied for the voltammetric response at the GC/MWCNT-SnO\(_2\) electrode. However, in the presence of levofloxacin, the GC/MWCNT-SnO\(_2\) electrode (curve c) exhibited a well-defined oxidation peak at a potential value of +0.91 V. This oxidation process is attributed to the irreversible oxidation of the piperazine group of the levofloxacin molecule. The same oxidation process was observed at an identical potential value for GC electrode modified with MWCNT in the absence of SnO\(_2\) rods (curve b). Therefore, the GC/MWCNT-SnO\(_2\) electrode presented higher anodic current intensity for levofloxacin in comparison with the current observed for the electrode GC/MWCNT in the absence of SnO\(_2\) rods. The levofloxacin oxidation process on the GC/MWCNT-SnO\(_2\) electrode showed an increase by a factor of 2.7-fold in the current peak. Comparing the GC/MWCNT-SnO\(_2\) electrode with the bare GC (curve a), the proposed sensor exhibited an increase of 4.9-fold in current peak. In addition, it was observed a shift in the oxidation potential value in 115 mV for more negative values. The increase in current value reflects the increase of the electroactive surface area by the formed MWCNT-SnO\(_2\) rods composite. Such properties make the GC/MWCNT-SnO\(_2\) composite electrode an interesting setup for electrochemical sensing.

A pH study was performed in order to evaluate the mechanism of levofloxacin oxidation at the GC/MWCNT-SnO\(_2\) surface. The relationship between the levofloxacin oxidation potential and the pH was studied by DPV experiments. The pH values was studied in a range varying from 3.0 to 9.0 in 0.2 mol L\(^{-1}\) PBS containing 100 µmol L\(^{-1}\) of levofloxacin. The results presented in Figure 4 show a plot of the DPV current peak (\(I_{pa}\)) and the potential peak (\(E_{pa}\)) as a function of pH. The variation of \(E_{pa}\) with pH can provide valuable information about the levofloxacin oxidation process.

Reducing the hydrogen ionic concentration of the electrolyte causes a shift in peak potential towards more negative values, as illustrated in Figure 4. This is due to the deprotonation of the antibiotic molecule. The oxidation process was facilitated at higher pH value. The \(E_{pa}\) vs. pH showed a linear relationship, with a slope of 60 mV per pH unit. Thus, an electrochemical process involving the same number of protons and electrons during the electrooxidation of levofloxacin can be proposed. The slopes for levofloxacin are close to that expected for a two electron electrode reaction, which is 59.2 mV per pH unit at 25 °C. The number of protons transferred is probably two, which corresponds to 59.2 (\(h/n\)) mV per pH unit; where \(h\) and \(n\) are the number of protons and electrons, respectively, involved in the electrode process. Therefore, the oxidation process proposed for levofloxacin involves two electrons and two protons, in agreement with the work described by Wen et al.\(^{19}\)

The plot of \(I_{pa}\) vs. pH for levofloxacin shows that the anodic peak current increased significantly when the pH was changed from 3.0 to 5.0, remained practically constant from 5.0 to 6.0 and then decreased at higher pH values. This value was expected, given that the \(pK_a\) of the levofloxacin is 5.5 for carboxylic acid group.\(^{24}\) Therefore, pH 6.0 was chosen to be used in subsequent experiments.
Effect of the electrode composition

The influence of the composition of the electrode material on the voltammetric response of the GC/MWCNT-SnO$_2$ electrode was evaluated by DPV in 0.1 mol L$^{-1}$ PBS at pH 6.0 containing 100 µmol L$^{-1}$ of levofloxacin. For the electrode composition study, the amount of MWCNT was fixed and the quantity of Sn was varied in the following proportions: 10%, 20%, 25%, 30%, and 40% (m/m). In Figure 5, it was observed that the anodic current peak increased with the amount of Sn in the composite up to 20% (m/m), decreasing when high amounts of Sn was used. This composition was then used for preparation of all electrodes used for levofloxacin analysis. This behaviour is probably due to the formation of SnO$_2$ clusters when more SnO$_2$ is deposited. Based on these results, 20% (m/m) of Sn was used in all electrodes prepared for levofloxacin analysis.

Analytical characteristics

Using DPV experiments, with a scan rate of 10 mV s$^{-1}$, pulse amplitude of 100 mV and a step potential of 2 mV, the proposed GC/MWCNT-SnO$_2$ electrode was applied to investigate the electrochemical response as a function of the levofloxacin concentration. All measurements were made in triplicate and the results are indicated as the mean value. The analytical response shown in Figure 6 has a linear response in the range from 1.0 to 9.9 µmol L$^{-1}$, in accordance with the following equation:

\[
I_{pa} (\mu A) = 0.18 (\mu A) + 0.36 (\mu A \text{ µmol L}^{-1}) \text{[levofloxacin]} \text{ (µmol L}^{-1})
\]  

with a correlation coefficient of 0.998 ($n = 10$). The limit of detection (LOD) obtained was 0.2 µmol L$^{-1}$ (72.0 mg L$^{-1}$), being determined using a $3\sigma$ / slope ratio, where $\sigma$ is the standard deviation of the mean value for 10 voltammograms of the blank.

Comparing the results at the GC/MWCNT-SnO$_2$ electrode with the few reports of electrochemical methods for levofloxacin detection, higher detection limits of 1.0 µmol L$^{-1}$ and 4.0 × 10$^{-7}$ mol L$^{-1}$ were observed using a poly(o-aminophenol)/MWCNT composite film and a MWCNT-polymeric alizarin film modified electrode, respectively. A similar LOD value was reported by Radi et al. Using other analytical methods for levofloxacin determination, such as electrophoresis, HPLC and liquid chromatography-tandem mass spectrometry system (LC-MS/MS), the detection limits of 1.02 mg L$^{-1}$ (2.8 µmol L$^{-1}$), 0.25 mg mL$^{-1}$ (0.7 µmol L$^{-1}$) and 3.6 ng g$^{-1}$ (1.0 × 10$^{-8}$ mol L$^{-1}$) were obtained, respectively. Although lower detection limits could be observed, the electrochemical methods have the advantage of low cost, easy operation, potential for miniaturization and automation, construction of simple portable devices for fast screening purposes and in-field/on-site monitoring.

The reproducibility of the GC/MWCNT-SnO$_2$ electrode was measured from seven experiments, in which each experiment consisted of five sequential DPV voltammograms. These experiments were performed on different days. Prior to each experiment, the electrode surfaces were rinsed thoroughly with double-distilled water. Thus, the DPV voltammograms were performed in 0.1 mol L$^{-1}$ PBS at pH 6.0 containing 100 µmol L$^{-1}$ levofloxacin. The relative standard deviation (RSD) was calculated as 1.9%. In addition, intra-assay precision tests were performed from ten DPV voltammograms of that same solution. The RSD was found to be 1.5%.
Selective determination of levofloxacin in the presence of interferents

The ability to determine levofloxacin in the presence of ascorbic acid (AA) and uric acid (UA) was investigated. In all DPV experiments (Figure not shown) it was not observed an overlap process of the levofloxacin oxidation peaks and the oxidation peaks of the interfering substances studied. In addition, the large separation of the peak potentials allows the selective and simultaneous determination of AA, UA and levofloxacin in the mixture. The oxidation peak potentials of AA, UA and levofloxacin are well-resolved at GC/MWCNT-SnO$_2$ electrode with the peak potentials at +0.17, +0.53, +0.91 V, respectively. The effect of AA and UA in the levofloxacin anodic peak current was evaluated using 0.1 mol L$^{-1}$ PBS at pH 6.0 containing a fixed concentration of 50 µmol L$^{-1}$ of levofloxacin, and sequential additions of 25, 50 and 100 µmol L$^{-1}$ of AA and UA. Recoveries between 98.8 and 102.5% of levofloxacin (n = 3) were obtained, for 25, 50 and 100 µmol L$^{-1}$ of AA added to each measurement, and recoveries between 97.6 and 99.3% of levofloxacin (n = 3) were obtained, for 25, 50 and 100 µmol L$^{-1}$ of UA added to each measurement. In the AA and UA concentration range studied it was observed that the decrease or increase in levofloxacin height peak was negligible. Also, the interfering substances studied did not shift the levofloxacin oxidation peak, indicating that the analytical signal does not suffer interference of AA and UA.

Conclusions

A novel electrode was developed using MWCNT-SnO$_2$ rods composite, which can be used for the levofloxacin detection, being a promising alternative for use in environmental analysis. The MWCNT-SnO$_2$ composite was successfully characterised by EDX, SEM and electrochemical techniques, which indicated that the SnO$_2$ rods were dispersed in the MWCNT. Regarding the use of GC as the working electrode in DPV measurements, the GC/MWCNT-SnO$_2$ composite electrode improved the current peak almost 5-fold for the levofloxacin oxidation. Finally, the synergetic effect of the carbon nanotubes and SnO$_2$ rods yielded lower LOD and improved the reproducibility, repeatability, and the sensitivity of the composite electrode, which augurs well for future applications in this area.

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