Int. J. Electrochem. Sci., 7 **(2012)** 11745 - 11757

International Journal of ELECTROCHEMICAL SCIENCE
www.electrochemsci.org

Amperometric and Voltammetric Determination of Oxytetracycline in Trout Salmonid Muscle Using Multi−wall Carbon Nanotube, Ionic Liquid and Gold Nanoparticle Film Electrodes

Edgar Nagles[1,2,*], *Paola Alvarez*[2], *Verónica Arancibia*[1], *Mauricio Baez*[2], *Virginia Garreton*[2], *Nicole Ehrenfeld*[2].

[1] Pontificia Universidad Católica de Chile, Facultad de Química, Vicuña Mackenna 4860, Santiago–7820436, Chile.
[2] Austral Biotech, Santiago, Chile.
[*]E-mail: ernagles@uc.cl

Received: 14 October 2012 / *Accepted:* 8 November 2012 / *Published:* 1 December 2012

The use of a multi−wall carbon nanotube, ionic liquid and gold nanoparticle film glassy carbon electrode to determine oxytetracycline (OTC), one of the mostly used antibiotics in salmon industry, by amperometry and adsorptive stripping voltammetry (AdSV) is reported. Electrodes were coated with $[BMIM]3F_5EPF_3$, $[EMIM]F_3MSO_3$, $[BMIM]PF_6$ and $[BMIM]BF_4$, achieving the highest sensitivity with $[BMIM]BF_4$. Using AdSV, variables like pH, scan rate (ν), accumulation potential, and time (E_{acc}, t_{acc}) were optimized. The best experimental conditions were: pH = 7.0; ν = 0.60 V s^{-1}; E_{acc} = 0.40 V, and t_{acc} = 70 s. The relative standard deviation obtained was 1.5% (n=5) for a solution containing $5.0x10^{-6}$ mol L^{-1} of OTC. Using AdSV and amperometric techniques, the linear calibration curves ranged up to $8.0x10^{-6}$ mol L^{-1} and the detection limits (3σ) were $1.5x10^{-7}$ and $2.0x10^{-8}$ mol L^{-1}, respectively. The methods were validated using fish tissue spiked with OTC. Because the sensitivity of the amperometric technique is higher than AdSV, the analysis of fish tissue samples was made using the former technique, getting a LoD of $5.3x10^{-10}$ mol L^{-1}.

Keywords: Oxytetracycline determination; Fish tissue analysis; Amperometric determination; Adsorptive stripping voltammetry; Multi−wall carbon nanotube−ionic liquid−gold nanoparticle film electrode.

1. INTRODUCTION

Oxytetracycline ((4S, 4aR, 5S, 5aR, 6S, 12aS)-4-dimethylamino-1,4,4a,5,5a,6,11,12a-octahydro-3,5,6,10,11,12a-hexahydroxy-6-methyl-1,12-dioxo-1,4,4a,5,5a,6,12,12aoctahydrotetracene-2-carboxamide) is one of the major antibacterial agents used since the early 1950s in animals and

humans because of their broad antibacterial spectrum activity, including gram−positive and gram−negative bacteria. Nevertheless, they continue to be used because of their simplicity and low cost. Antibacterial drugs such as tetracycline (TC), chlortetracycline (CTC), lymecycline (LMC) and doxycycline (DC) with similar behavior are used in a variety of food−producing animals including cattle, sheep, goats, pigs, poultry, and fish. OTC, TC, and CTC are natural tetracyclines (TCs) obtained by fermentation with the soil actinomycete *Streptomyces rimosus*. These substances can be absorbed by humans through the animals treated with these drugs, causing serious threat to human health such as allergies, toxic effects, and bacterial resistance [1−3]. The European Union has stated a maximum residue limit of 100 μg kg^{-1} for total TCs in edible animal tissues [4]. Salmonid industry is particularly sensitive on this regards and perform extensive sampling of the fishes to check absence of antibiotics before hatchering. The development of new methods for quantifying traces of these drugs is required.

Several methods have been reported for the analysis of TCs in different matrices, such as HPLC coupled with different detectors: classic UV [5], UV−DAD [6], AD [7,8], FD [9] and MS [10,11]. Önal et al. [12] reported limits of detection (LoD) of 4.0; 0.9; 1.0 and 0.1 μg kg^{-1} obtained with HPLC coupled with classic UV, UV−DAD, FD and MS−MS detectors, respectively. Recently, capillary electrophoresis [13], potentiometric ISEs or sensors [14] and immunochemical methods coupled with photometric and fluorometric detections [15] have been used for the determination of OTC, giving LoD of 61, 30, 16 and 0.08 μg kg^{-1}, respectively. On the other hand, efforts have been performed for quantifying TCs by electroanalytical techniques, due to the low detection limits, selectivity and relatively inexpensive instrumentation. In these, different electrodes such as HMDE [16−18], gold nanoparticles [19], screen−printed gold [20,21], boron−doped diamond [22] and carbon fiber microelectrodes [23] were shown to be useful for OTC and/or other TC determinations.

The aim of this study was to optimize AdSV and amperometric techniques to determine OTC using a glassy carbon electrode (GCE) modified with carbon-wall nanotubes and gold nanoparticle film. For enhancing the adsorptive process and the sensitivity of the method, some ionic liquids (Ils) were included in the electrode surface. Importantly, ionic liquids have high conductivity, high chemical and thermal stability, almost negligible vapor pressure and wide electrochemical potential windows [24−26]. These Ils carrying long–chain alkyl groups may be adsorbed easily onto the working electrode by hydrophobic and electrostatic attraction, and the OTC in the solution can be attracted by the ionic liquid and transported to the electrode surface in the accumulation step.

2. EXPERIMENTAL PART

2.1. Apparatus

Amperograms and adsorptive stripping voltammograms were obtained using a BASI CV50W system in a three–electrode configuration. A modified glassy carbon electrode with multi−wall carbon nanotubes, an ionic liquid and gold nanoparticle film (MWCNT−IL−AuNP−GCE) was used as working electrode (∅ 3 mm). Ag/AgCl/KCl/3 mol L^{-1} and platinum wire were used as reference and auxiliary electrodes, respectively. The pH measurements were made with an Orion–430 digital pH/mV meter equipped with combined pH glass electrode. A multivortex was used in the extraction of OTC from the fish samples.

2.2. Chemicals

Water used for sample preparation, dilution of reagents, and rinsing purposes was obtained with a Milli–Q system (18.2 Ohm. Millipore, USA). Potassium ferricyanide, nitric acid, phosphoric acid, acetic acid and chloroauric acid solution (1000 mg L^{-1}) were analytical grade, while methanol, acetonitrile, dichloromethane and hexane were HPLC grade (Merck). The stock solutions of oxytetracycline, chlortetracycline, doxycycline and lymecycline (Sigma−Aldrich) were freshly prepared each working day by dissolving the reagents in methanol (0.5 mmol L^{-1}). Chitosan (low weight) and the multi−walled carbon nonotubes used (MWCNTs, ∅ 5−10 nm, length 0.5−20 μm, purity: ≥ 95%) were obtained from Sigma−Aldrich. Ionic liquids: 1–ethyl–3–methylimidazolium trifluoromethanesulfonate ([EMIM]F_3MSO_3), 1–butyl–3–methylimidazolium tetrafluoroborate ([BMIM]BF_4), 1–butyl–3–methylimidazolium hexafluorophosphate ([BMIM]PF_6), and 1–butyl–3–methylimidazolium trispentafluoroethyltrifluorophosphate ([BMIM]$3F_5EPF_3$) were purchased from Merck. Phosphate buffer solutions were used to investigate pH in the 2.5–7.4 range. These buffers were prepared with 0.10 mol L^{-1} phosphoric acid solution, adjusting to the required pH with NaOH solution (0.5 mol L^{-1}).

2.3. Preparation of modified MWCNT−IL−AuNP glassy carbon electrode

Before measurement, the glassy carbon substrate electrode was thoroughly polished using a polishing pad with 0.3 and 0.05 μm Al_2O_3 slurry, rinsed with 0.3 mol L^{-1} HNO_3, water and methanol for five minutes in an ultrasonic bath, and dried with N_2. 2.0 mg of MWCNTs composites were dispersed in 1.0 mL of chitosan solution (1% acetic acid) and sonicated for 90 min. 10–μL of composite solution or 10–μL of MWCNTs–chitosan solution was placed on the electrode surface and the solvents were evaporated at room temperature for 15 min. Then 10 μL of concentrated ionic liquid was placed on the surface of the recently modified electrode, and the solvents were evaporated at 70 ^oC and at room temperature (60 min). The electrode was washed to remove excess solvent and it was then transferred to the plating solution containing 200 mg L^{-1} $HAuCl_4$ and electrodeposited gold nanoparticles (AuNPs) at 0.20 V for 60 s [27]. The modified MWCNTs−IL−AuNP glassy carbon electrode was submitted to 20 cycles of potential between 0.00 to 1.20 V (0.10 mV s^{-1}) to obtain a stable, reproducible and clean surface. The same electrode was used in a series of measurements.

2.4. Trout muscle sample preparation

Seven trouts treated with OTC were obtained from salmonid farms in Chiloe Island (Chile). The extraction was carried out by the method reported by Ueno [18] from 5.0 g of fish muscle homogenized in a Multivoltex (Heidolph, Germany).

2.5. Measurement Procedure

9.0 mL of deionized water, 500 μL of phosphate buffer solution (0.1 mol L^{-1}) and 10 μL aliquots of OTC solution (5 mg L^{-1}) were pipetted into the electrochemical cell. The solution was purged with nitrogen (saturated with water vapor) for 300 s in the first cycle and for 60 s in each successive cycle, for a given accumulation potential and time at a stirring speed of 500 rpm. After an equilibration time of 10 s, adsorptive voltammograms were recorded, while the potential was scanned from 0.0 to 1.2 V. Each voltammogram or amperogram was repeated three times. Calibration curves were obtained and linear regression and limits of detection were calculated. The proposed method was applied to the determination of OTC in trout muscle samples. To eliminate matrix effects the standard addition method was used. All data were obtained at room temperature (~25 °C).

3. RESULTS AND DISCUSSION

3.1. Characterization of the modified MWCNT−IL−AuNP glassy carbon electrode with an $Fe(CN)_6^{3-}$ solution.

Preliminary experiments were carried out to identify the general features that characterize the surface of the electrodes using an $Fe(CN)_6^{3-}$ solution.

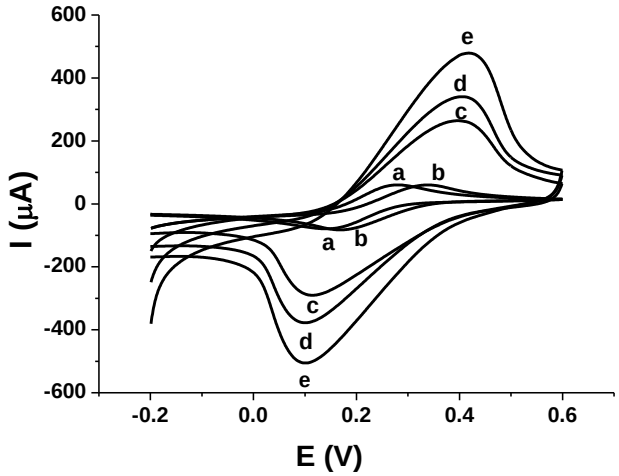

Figure 1. CV of $FeCN)_6^{3-}$ solution (10.0 mmol L^{-1}) in pH 7.0 phosphate buffer using GCE (curve a), AuNPs−GCE (curve b), MWCNTs−GCE (curve c), MWCNT−AuNP−GCE (curve d) and MWCNT−IL−AuNP−GCE (curve e). Scan rate (ν) 100 mV s^{-1}.

Figure 1 shows cyclic voltammograms (CVs) of $Fe(CN)_6^{3-}$ solution (10.0 mmol L^{-1}) at 100 mV s^{-1} in phosphate buffer solution at pH 7.0, using GCE (curve a), AuNPs−GCE (curve b), MWCNTs−GCE (curve c), MWCNT−AuNP−GCE (curve d) and MWCNT−IL−AuNP−GCE (curve e) electrodes. CVs obtained with all the electrodes showed a quasi−reversible oxidation processes between 0.27 to 0.42 V and the corresponding reduction between 0.18 to 0.10 V. Under these

conditions the ipc/ipa ratio was closed to 1 (curves a−e). Peak current is low when only AuNPs are electrodeposited on the GCE (curve b), but it increased twice when the GCE was modified with MWCNTs, but the process was less reversible (ΔEP = 300 mV) (curve c). When gold nanoparticles were deposited on the GCE modified with multi−wall carbon nanotubes (MWCNT−AuNP−GCE) the peak current of the $Fe(CN)_6^{3-}$ oxidation/reduction process continued increasing and the peak potential was not displaced (curve d). Finally, when gold nanoparticles were deposited on the GCE modified with multi−wall carbon nanotubes and $[BMIM]BF_4$ (MWCNT−IL−AuNP−GCE) the peak current of OTC oxidation was the highest (curve e). The presence of the ionic liquid favored the charge transport.

3.2. Study of the influence of ionic liquid type with $Fe(CN)_6^{3-}$ solution.

Figure 2 shows the CV of $Fe(CN)_6^{3-}$ solutions (5.0 mmol L^{-1}) at 100 mV s^{-1} in pH 7.0 phosphate buffer using MWCNTs−AuNPs film electrodes coated with different ionic liquids: $[BMIM]3F_5EPF_3$ (curve a), $[EMIM]F_3MSO_3$ (curve b), $[BMIM]PF_6$ (curve c) and $[BMIM]BF_4$ (curve d). An almost imperceptible signal for the oxidation/reduction process of $Fe(CN)_6^{3-}$ was obtained with the electrode coated with $[BMIM]3F_5EPF_3$ (curve a), while the highest peak current was obtained when the electrode was coated with $[BMIM]BF_4$ (curve d), indicating the pre-concentration of $Fe(CN)_6^{3-}$ on the electrode surface. The charge transport was affected by the anionic component in this order: $3F_5EPF_3^- < F_3MSO_3^- < PF_6^- < BF_4^-$. The molecular volume of BF_4^- is smaller than that of PF_6^- and it has higher conductivity [29]. However, Gou et al. [30] determined TCs using an MWCNTs-GCE modified with $[BMIM]PF_6$, getting good results. $[BMIM]BF_4$ showed the best performance, so it was chosen as the optimum for this study.

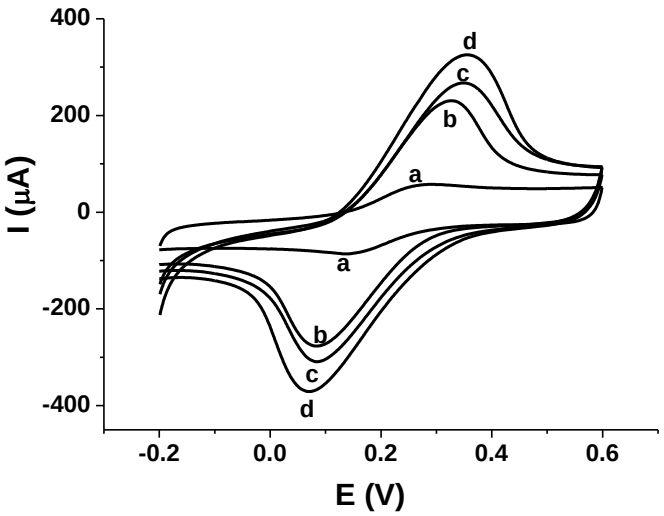

Figure 2. CV of $FeCN)_6^{3-}$ solution (5.0 mmol L^{-1}) in pH 7.0 phosphate buffer using a modified MWCNTs−AuNPs-GCE coated with $[BMIM]3F_5EPF_3$ (curve a), $[EMIM]F_3MSO_3$ (curve b), $[BMIM]PF_6$ (curve c) and $[BMIM]BF_4$ (curve d). Scan rate (ν) 100 mV s^{-1}.

3.3. Electrochemical behavior of OTC using different electrodes.

The electrochemical oxidation of OTC involves the phenol groups generating protons and the potential decrease when the pH of solution increases. Figure 3 shows the CV of OTC (40.0 μmol L^{-1}) at 100 mV s^{-1} in pH 7.0 phosphate buffer, using GCE (curve a), AuNPs−GC (curve b), MWCNTs−GC (curve c), MWCNT−AuNP−GC (curve d) and MWCNT−IL−AuNP−GC (curve e) electrodes. When GC and AuNPs−GC electrodes were used, the CV did not show any signal of an OTC oxidation/reduction process (curves a, b). With the GCE modified with MWCNT the peak current was very small (curve c). However, when MWCNT−AuNP−GCE was used the peak current increased and two oxidation peaks at 0.73 and 0.91 V were seen (curve d). On the other hand, when the electrode was modified with [BMIM]BF_4 the peak current of OTC (0.76 V) increased considerably and the reduction peak was seen at 0.51 V. Therefore, it can be concluded that with the addition of [BMIM]BF_4 on the electrode surface the process is more reversible and the method is more sensitive.

It have been reported that tetracycline is oxidized at 0.54 V in pH 7.0 phosphate buffer solution using a multi-wall carbon nanotube–ionic liquid film coated glassy carbon electrode [30]. Whereas, OTC is irreversibly oxidized at about 1.2 V in sulfuric acid (0.01 mol L^{-1}) using a mixed-valence ruthenium oxide—ruthenium cyanide deposited on a glassy carbon electrode [31]. The same oxidation potential was obtained for OTC in potassium dihydrogen phosphate solution at pH 2 using a disposable screen-printed gold electrode [21]. On the other hand, according to their molecular structure OTC can also be studied by electrochemical reduction of carbonyl groups to hydroxyl groups at about -1.08 V (HMDE) [32].

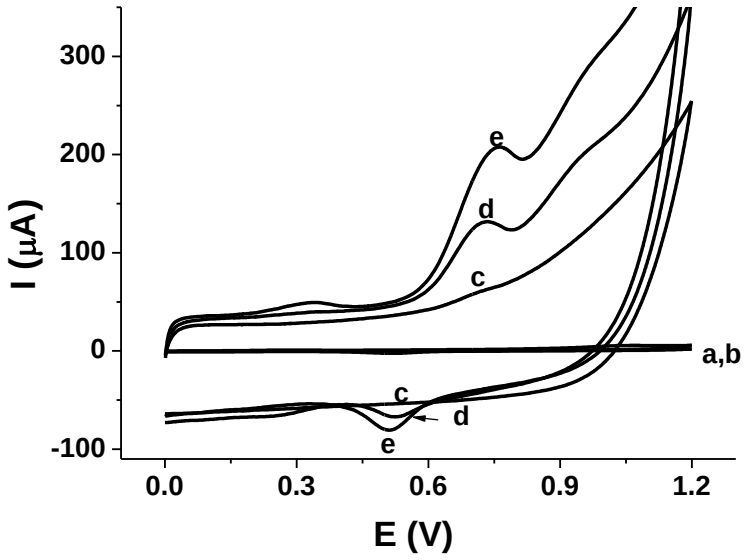

Figure 3. CV of OTC solution (40.0 μmol L^{-1}) in pH 7.0 phosphate buffer using different electrodes: GC (curve a), AuNPs−GC (curve b), MWCNTs−GC (curve c), MWCNT−AuNP−GC (curve d) and MWCNT−IL−AuNP−GC (curve e). Scan rate (v) 100 mV s^{-1}.

3.3. Electrochemical behavior of TCs with an MWCNT−IL−AuNP glassy carbon electrode.

Because TCs are grouped in a family that have similar structures with antibacterial activity, an electrochemical study of several TCs was carried out. Figure 4 shows the CVs of DOC (curve a), CTC (curve b), LMC (curve c), and OTC (curve d) solutions (40.0 μmol L^{-1}) at 100 mV s^{-1} in pH 7.0 phosphate buffer using a modified MWCNT−IL−AuNP−GC electrode. The highest peak current was obtained with OTC. Due to these results and the fact that OTC is the most widely used antibiotic in salmon industry, further studies were carried out only with OTC. With the purpose of obtaining higher sensibility with AdSV, studies to evaluate the effect of pH, accumulation time and potential (E_{acc}, t_{acc}) were made.

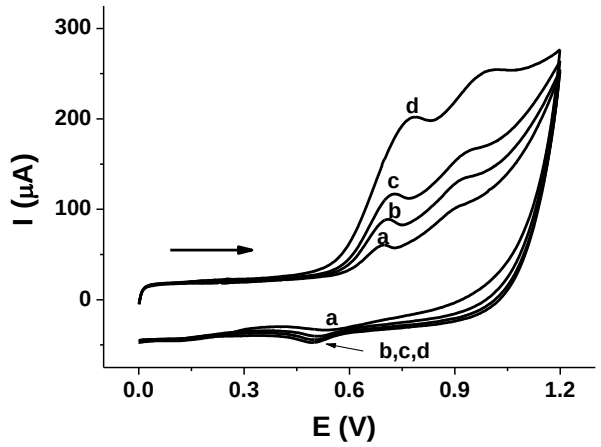

Figure 4. CV of Tcs solutions (40.0 μmol L^{-1}) in phosphate buffer pH 7.0 using a modified MWCNTs−IL−AuNPs-GC electrode. DOC (curve a), CTC (curve b), LMC (curve c) and OTC (curve d). Scan rate (ν) 100 mV s^{-1}.

3.4. Effect of pH variation

Besides de electrode, the buffer condition may influence highly the redox characteristics of OTCs, the electrochemical oxidation of OTC was studied in the 2.5–7.5 pH range (Fig. 5). In order to keep the buffer composition constant when studying the effect of pH, phosphate/phosphoric acid solutions (0.1 mol L^{-1}) were used. The experimental conditions were: OTC 10.0 μmol L^{-1}; E_{acc} = 0.1 V and t_{acc} = 60 s; scan rate (ν) = 100 mV s^{-1}. The results show that increasing pH up to 5.0 – 7.0 also increased the peak current. However, at higher pH values (≥ 8.0) the peak current decreased, due probably that OTC is negatively charged. It should be noticed that the pKa values of OTC are 3.3, 7.0, 9.0 and 13.0 [33]. pK_2 corresponds to the deprotonation of the dimethylamine group, and it is probably oxidized at around 0.76 V. Considering these results, pH 7.0 was used for further experiments. However, Yañez-Sedeño [3] reported that the oxidation currents of tetracyclines decreased with increasing pH and practically disappeared at pH values around 7. The similar form, Orawon et al [22] found that tetracycline in phosphate buffer of pH 2 provided the highest peak current.

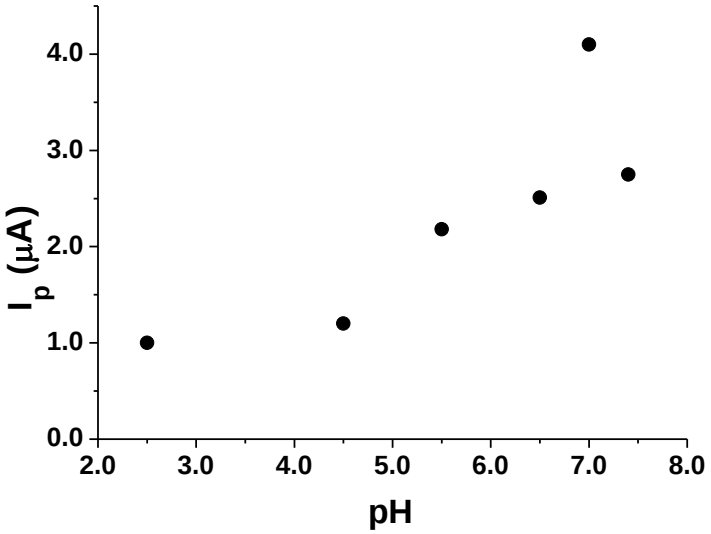

Figure 5. Effect of pH on the peak current of OTC solution (10.0 μmol L^{-1}). Conditions: E_{acc} 0.1 V; t_{acc} 60 s.

3.5. Effect of accumulation potential and time (E_{acc}, t_{acc}) on the peak current of OTC

The effect of the accumulation potential on the adsorptive peak current of OTC at pH 7.0 was studied from −0.1 to 0.6 V. The experimental conditions were: OTC 10.0 μmol L^{-1}; scan rate (ν) = 100 mV s^{-1}, and t_{acc} = 60 s. As shown in Figure 6A, the peak current of OTC increases up 0.40 V and decreases when the potential is changed from 0.50 to 0.60 V. An accumulation potential of 0.40 V gave the best sensitivity and was selected for further measurements. Masawat et al [21] studied the effect of applied potential from 0.8 to 1.3 V (pH 2) they observed that the electrode response was quite rapid and the peak current increased with increasing applied potential up to 1.2V.

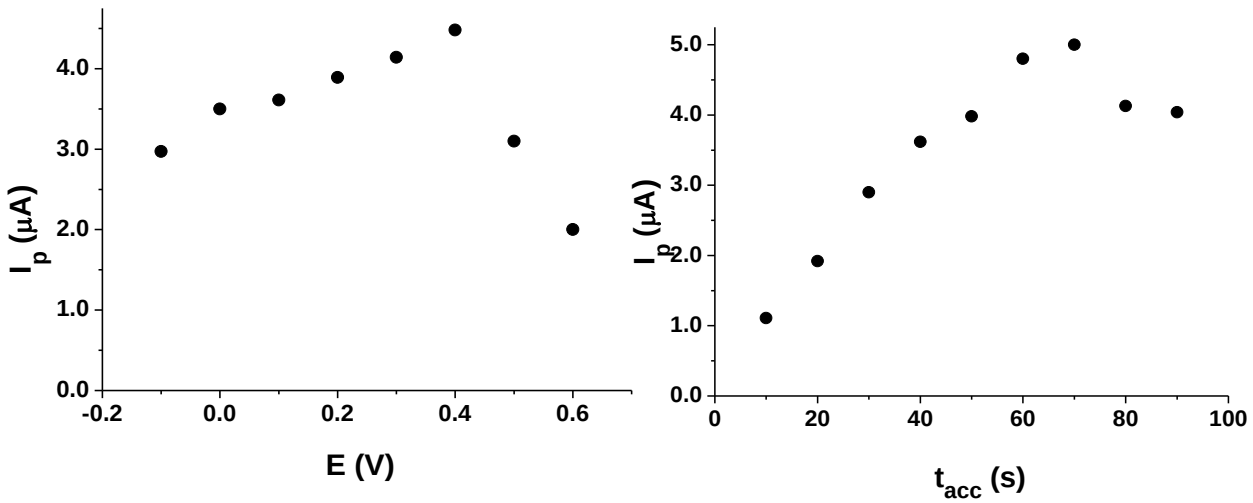

Figure 6. (A) Effect of accumulation potential on the peak current of OTC solution (10.0μmol L^{-1}). Conditions: pH 7.0; t_{acc} 60 s. (B) Effect of accumulation potential on the peak current of OTC solution (10.0 μmol L^{-1}). Conditions: pH 7.0; E_{acc} 0.40 V.

On the other hand, Figure 6B shows the effect of accumulation time on the adsorptive peak current of OTC at pH 7.0 over the 0–90 s range. The experimental conditions were: OTC 10.0 μmol L^{-1}; scan rate (ν) = 100 mV s^{-1}, and E_{acc} = 0.40 V. Peak current increases with increasing accumulation time, indicating that OTC is readily adsorbed on the modified electrode surface. Peak current of OTC become constant when t_{acc} reached 70 s, higher times probably producing saturation of the electrode surface. A t_{acc} of 70 s was used for further studies.

3.6. Effect of scan rate (ν) on the peak current of OTC

Figure 7 shows the effect of scan rate (ν) on the adsorptive peak current of OTC at pH 7.0, over the 5 – 100 mV s^{-1} range. Experimental conditions were: OTC 10.0 μmol L^{-1}; t_{acc} = 70 s, and E_{acc} = 0.40 V. The results shows that the peak current of OTC increases linearly with ν between 5 − 60 mV s^{-1} (R = 0.98), indicating that the electrochemical process is controlled by adsorption. On the other hand, with scan rates between 70 – 100 mV s^{-1} the peak current decreases, indicating that electron transfer is not fast. Therefore, a scan rate of 60 mV s^{-1} was used for further studies.

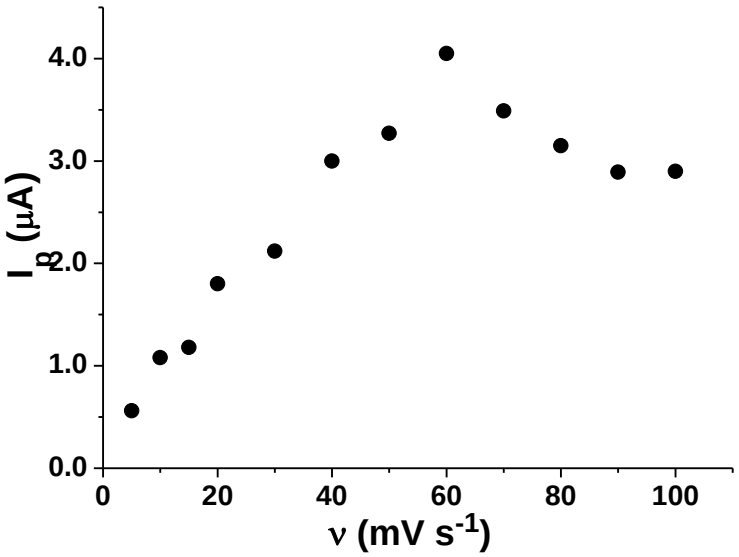

Figure 7. Effect of scan rate on the peak current of OTC solution (10.0 μmol L^{-1}). Conditions: pH 7.0; E_{acc} 0.40 V; t_{acc} 70 s.

3.7. Analytical parameters

Once settled the optimized conditions for OTC's current peak, two techniques were evaluated: Adsorptive Stripping Voltammetry and Amperometry.

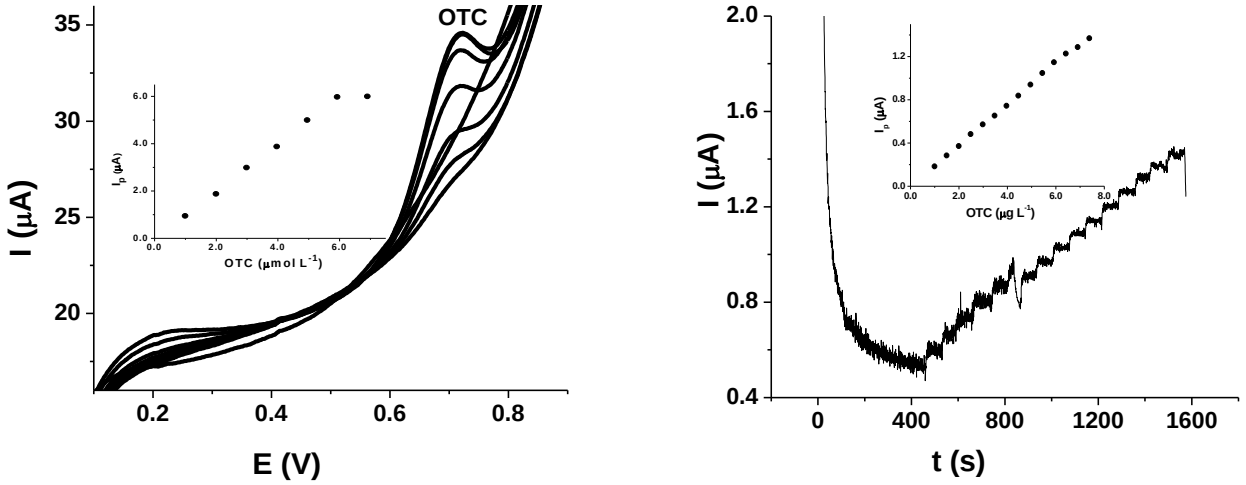

Figure 8. (A) AdSV of OTC solutions using an MWCNT−IL−AuNP−GC electrode. Conditions: pH 7.0; E_{acc} 0.4 V; t_{acc} 70 s, and scan rate (ν) 60 mV s^{-1}. (B) Amperometric response to OTC solutions at a potential of 0.74 V (pH 7.0; 500 rpm). The inset shows calibration curves.

AdV for the determination of OTC were obtained under the optimized conditions: pH 7.0 (0.1 mol L^{-1}), E_{acc} 0.4 V, t_{acc} 70 s, and scan rate (ν) 60 mV s^{-1} (Fig. 8A). This figure shows that the peak current of OTC increases proportionally as its concentration is increased between 0.9–6.0 μmol L^{-1}. The LoD (3σ) obtained was $1.5x10^{-7}$ mol L^{-1} and the relative standard deviation was 1.5% at $5.0x10^{-6}$ mol L^{-1} of OTC (n=5).

On the other hand, amperograms and the calibration curve are shown in Figure 8B, were the following conditions were used: pH 7.0; (0.1 mol L^{-1}); E 0.70 V. It is seen that the peak current of OTC increases proportionally with concentration between 0.2–9.0 μmol L^{-1}, achieving an LoD of $2.0x10^{-8}$ mol L^{-1}. When the concentration of OTC exceeded 10.0 μmol L^{-1} its peak current became almost constant, probably due to saturation of the electrode surface. The results presented until here show that the amperometric technique is more sensitive than AdSV, so the former was chosen for further studies with fish samples. These values were similar to others reported for tetracycline determination using different electrodes. For instance, Zhao et al [30] used a multi-wall carbon nanotube–ionic liquid glassy carbon electrode and got a LoD of ≈ $3x10^{-8}$ mol L^{-1}; Orawon et al [22] used nickel-implanted boron-doped diamond thin film electrode and got $1x10^{-8}$ mol L^{-1}; Shaidarova et al [31] used an electrode modified by a mixed-valence ruthenium oxide−ruthenium cyanide film for to determine tetracycline, oxytetracycline and doxycycline and got about $5x10^{-8}$ mol L^{-1}. Whereas, Yañez−Sedeño et al [3] developed an HPLC method with amperometric electrochemical detection for tetracycline, oxytetracycline, chlortetracycline and doxycycline using a multi-wall carbon nanotube electrode and got a LoD of $9x10^{-8}$ mol L^{-1} for OTC.

3.8. Validation of the method using the amperometric technique

To validate the current electroanalytical method with fish samples, we first analyzed OTC in fish meat samples (1.0 mL) spiked with 0.59 mg L^{-1} of OTC. The experimental conditions for the amperometric assays were: pH 7.0, E = 0.70 V. Figure 9 shows amperograms and the calibration curve. The value obtained using the standard addition method was 0.53 mg L^{-1} ± 0.01 mg L^{-1} (–10.1% RE), a value extremely similar to the one expected. The LoD (3σ) was $5.2x10^{-10}$ mol L^{-1}. This value is better than many LoD reported for tetracyclines analysis.

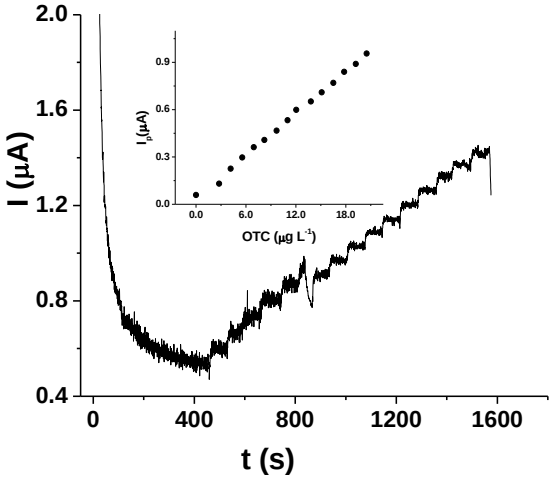

Figure 9. Amperometric response of the MWCNT−IL−AuNP−GC electrode to fish muscle sample spiked with 0.59 mg L^{-1} of OTC and successive additions of standard solution at a potential of 0.74 V (pH 7.0; 500 rpm). The inset shows calibration curve.

3.9. Analysis of OTC in real fish samples using the amperometric technique

The standardized method was applied to the determination of OTC in seven trout muscle samples that were treated with this drug in the field under standard feeding conditions of the salmon industry. OTC was found in only two samples and the concentrations were 2.0 ± 0.1 and 14.9 ± 0.2 mg kg^{-1} (Figure 10). To check the reliability of the method all seven samples were analyzed by HPLC−DAD, getting 1.8 and 14 mg kg^{-1}. The absence of detectable OTC in five of the samples is explained because those animals were treated several weeks before our analyses were made, and by that time the drug was no longer bioavailable. Those samples under HPLC limit of detection could be detected with the amperometric system, but very close to the noise (data not shown), therefore it was also assumed to be under the limit detection. Work has to be done to define sensibility, precision and limit of detection for this new amperometric system.

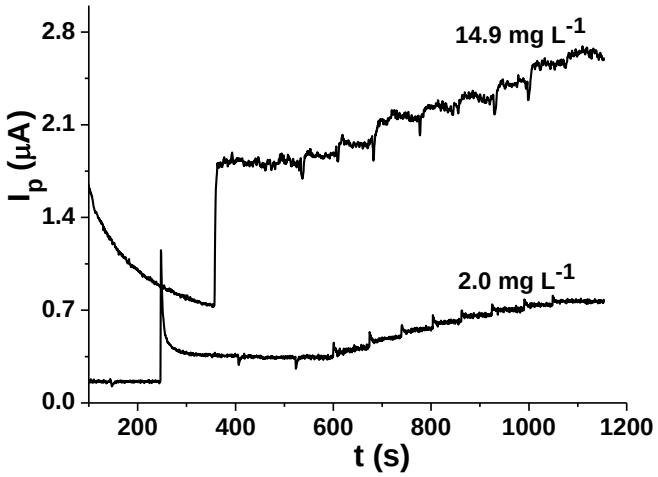

Figure 10. Amperometric response of the MWCNT−IL−AuNP−GC electrode to two real samples of trout salmonid muscle and successive additions of OTC standard solution at a potential of 0.74 V (pH 7.0; 500 rpm).

4. CONCLUSIONS

In the current work we have described a new method to quantify OTC, or other tetracyclines, using an amperometric technique whose sensitivity was significantly increased by including the ionic liquid [BMIM]BF_4. This method was successfully used to quantify OTC in a very complex food sample as trout, a salmonid fish whose muscle is full of several potentially interfering compounds like lipids, proteins, dyes, etc.

Two techniques were evaluated: amperometry and adsorptive stripping voltammetry. The former was more sensitive than the latter. The limit of detection achieved for OTC with AdSV is 1.5^{-7} mol L^{-1}, approximately ten times higher than that obtained with amperometry: $2.0x10^{-8}$ mol L^{-1} with synthetic solution and more of two hundred times than that obtained with real samples: $5.2x10^{-10}$ mol L^{-1}.

The determination of OTC was carried out on a modified electrode with carbon nano-particles coated with (EMIM)BF_4 and gold nanoparticles film. It is important to mention that this ionic liquid was very useful in the modification of the electrode because it increased the accumulation of OTC and therefore increased the sensitivity, and it was influential in the reversibility of the electrochemical reaction.

ACKNOWLEDGEMENTS
Financial support by FONDECYT under Postdoctoral Project 3120030 and INNOVA Project 09MCSS−6700 is gratefully acknowledged.

References

1. M. L. Nelson, S. B. Levy. *Annals of the New York Academy of Sciences*, 1241 (2011) 17.
2. Y. Ni, Sh. Li, S. Kokot, *Food Chem*., 124 (2011) 1157.
3. D. Vega, L. Agüi, A. Gonzáles−Cortés, P. Yánez−Sedeño, J.M. Pingarrón, *Anal. Bioanal. Chem*., 389 (2007) 951.
4. *Official Journal of the European Communities* L224, of 18 August 1990, Council Regulation 2377/90/EC; consolidated version of the Annexes I to IV updated up to 27.02.2009 obtained from http://www.emea.europa.eu/htms/vet/mrls/s.htm
5. D. J. Fletouris, E. P. Papapanagiotou, *Anal. Bioanal. Chem*., 391 (2008) 1189.
6. J. B. Lee, H. H. Chung, Y. H. Chung, K. G. Lee, *Food Chem*., 105 (2007) 1726.
7. A. Guzmán, L. Agui, M. Pedrero, P. Yañez-Sedeño, J. M. Pingarrón, *J. Pharmaceut. Biomed*., 33 (2003) 923.
8. I. G. Casella, F. Picerno, *J. Agr. Food Chem*., 57 (2009) 8735.
9. P. Penido, S. Rath, F. G. Reyes, *Food Chem*., 109 (2008) 212.
10. M. T. Meyer, J. E. Bumgarner, J. L. Varns, J. V. Daughtridge, E. M. Thurman, K. A. Hostetler, *Sci. Total Environ*., 248 (2000) 181.
11. W. Ben, Z. Qiang, C. Adams, H. Zhang, L. Chen, *J. Chromatogr., A* 1202 (2008) 173.
12. A. Önal, *Food Chem*., 127 (2011) 197.
13. J. M. Miranda, J. A. Rodríguez, C. A. Galán, *J. Chromatogr. A*, 1216 (2009) 3366.
14. X. X. Sun, X. Zhang, H. Y. Aboul−Enein, *Il Farmaco*, 59 (2004) 307.
15. C. Cháfer−Pericása, A. Maquieira, R. Puchadesa, J. Miralles, A. Moreno, N. Pastor−Navarro, F. Espinós, *Anal. Chim. Acta*, 662 (2010) 177.
16. M. A. Ghandour, A. M. M. Ali, *Anal. Lett*., 24 (1991) 2171.
17. E. Pinilla-Gil, L. Calvo-Blazquez, R. M. García−Monco, A. Sanchez-Misiego, *Fresenius Z. Anal*., Chem. 332 (1988) 821.
18. J. Wang, P. Peng, M. S. Lin, *Bioelectrochem. Bioenerg*., 15 (1986) 147.
19. P. Noruozi, M. R. Ganjali, P. Daneshgar, *Turk. J. Chem*., 31 (2007) 279.
20. Y. J. Kim, Y. S. Kim, J. H. Niazi, M. B. Gu, *Bioprocess Biosyt. Eng*., 33 (2010) 31.
21. P. Masawat, J. M. Slater, *Sensor Actuat. B*, 124 (2007) 127.
22. T. Surudee, C. Suchada, E. Yasuaki, S. Rika, C. Orawon, *Anal. Sci*., 21 (2005) 531.
23. L. Aguí, A. Guzmán, M. Pedrero, P. Yañez−Sedeño, J. M. Pingarrón, *Electroanalysis*, 15 (2003) 601.
24. P. A. Suarez, V. M. Selbach, J. E. Dullius, S. Einloft, C. M. Piatnicki, D. S. Azambuja, R. F. de Souza, J. Dupont, *Electrochim. Acta*, 42 (1997) 2533.
25. P. Brown, C. P. Butts, J. Eastoe, D. Fermin, I. Grillo, H. Lee, D. Parker, D. Plana, R. M. Richardson, *Langmuir*, 28 (2012) 2502.
26. P. D. Galgano, O. A. El Seoud, *J. Colloid Interface Sci*., 345 (2010) 1.
27. Ch. Xie, H. Li, Sh. Li, J. Wu, Zh. Zhang, *Anal. Chem*., 82 (2010) 241.
28. R. Ueno, K. Uno, S. S. Kuboda, Y. Horiguchi, *Nippon Suisan Gakk*., 55 (1989) 1273.
29. N. Guzmán, J. Fernández, M. Parada, C. Orbegozo, M. Rodríguez, A. Padrón, *Quim. Nova*, 33 (2010) 1703.
30. G. Guo, F. Zhao, F.Xiao, B. Zeng, *Int. J. Electrochem. Sci*., 4 (2009) 1365.
31. L. G. Shaidarova, A. V. Gedmina, I. A. Chelnokova, G. K. Budnikov, *Pharm. Chem. J*., 42 (2008) 545.
32. Y. Ni, S. Li, S. Kokot, *Food Chem*., 124 (2011) 1157.
33. N. Arnaud, J. Georges, *Analyst*, 126 (2001) 694.