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Square Wave Voltammetry in the Determination of Ni^{2+} and Al^{3+} in Biological Samples

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In this contribution, the amounts of Ni (nickel) and Al (aluminum) in tilapias (*Oreochromis niloticus*) were determined using square wave voltammetry (SWV) with glassy carbon working microelectrode with a mercury thin film, platinum counter electrode, and Ag/AgCl reference electrode. Ni was studied through the formation of the dimethylglyoxime-Ni (Ni-DMG) complex, while Al was studied through the formation of the Alizarin R-Al complex. The detection limit found for Ni-DMG and Alizarin R-Al complexes were 1.70×10^{-7} and 1.0×10^{-8} mol L^{-1}, respectively. The voltammetric anodic curves for the Alizarin R-Al complex were recorded over the potential range from –0.8 to –0.05 V while the voltammetric cathodic curve for the Ni-DMG complex was recorded over the potential range from –0.7 to –1.2 V. These methods detected low concentrations of Ni and Al in biological samples efficiently.

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Introduction

Natural bodies of water and river flows polluted with heavy metals discharged by industries are major contributors to human illness.[1] Heavy metals are available all over the word in either natural or synthetic form.[2] Ni^{2+} (nickel) is a toxic element; it is the 24th most abundant element on the earth crust, and it makes up 6% of center crust.[3] It is found in natural bodies of water and is an abundant biosphere component. The levels of Ni^{2+} in polluted areas range from 1 to 10 μg L^{-1} natural water. However, in some areas, Ni^{2+} concentrations may be higher because of volcanism.[4] The levels of Ni^{2+} in wastewater in industrialized areas are higher than the average levels of other areas.[5]

Ni^{2+} is also widely found in jewels. Approximately 10 to 15% people are sensitive to skin contact with this element and may suffer from illnesses such as dermatitis, eczema, and asthma. Daily intake of Ni^{2+}-enriched food is a major source of intoxication.[6]

Al^{3+} (aluminum) toxicity is associated with some clinical complications and neurological dysfunctions such as Alzheimer's disease, and it may affect the treatment of chronic renal illnesses.[7,8] Dialysis patients exposed to high Al^{3+} concentrations may exhibit dialysis encephalopathy and bone mineralization disorders such as dialysis osteodystrophy.[9] The rate of Alzheimer's disease is reportedly higher in places where the amounts of Al^{3+} exceed 0.1 mg L^{-1} than in places with amounts lower than 0.01 mg/L.[10]

Nowadays, considerable efforts are made to develop methods to determine trace heavy metals in biological and soil samples.[1] Modified solid microelectrodes with mercury thin film and mercury drop electrodes allow the direct determination of some metals with high sensitivity. However, metals like Al^{3+} need a complexing agent because their high oxidation potential and secondary currents affect the observation of the reduction peak. Square wave voltammetry (SWV) is very sensitive to a variety of trace metals on solid microelectrodes with mercury thin film.[4] It involves the complexation of trace metals with metal-specific ligands and the adsorption of the resulting complex onto the mercury surface film.[4] Since this is a surface technique, it is suitable for determining ultra-trace levels of metals in solutions such of Ni^{2+} and Al^{3+} using complexing agents. Voltammetric methods are used in environmental, food, and pesticide analysis. In addition, the modification of the surface of solid electrodes allows researchers to study problems related to metallothioneins (MT), which are formed by the interaction of metal ions with either amino acid or protein sulfydryl groups, with good sensitivity.[10]

The determination of Ni^{2+} and Al^{3+} in biological samples is very important to monitor their effects on tissues and the environment.[11] This work developed a voltammetric method to determine Ni^{2+} and Al^{3+} in tilapia samples through the formation of Ni-DMG and Alizarin R-Al complexes.

Materials and Methods

Materials

Standard solutions of either Ni^{2+} or Al^{3+} and DMG (dimethylglyoxime) and Alizarin (1,2-dihydroxy-9,10-anthracenedione) were purchased from Merck. Tilapias, *Oreochromis niloticus*, samples were contaminated using commercial feed previously enriched with either Ni^{2+} or Al^{3+} standard solutions. Two tanks (A and B) were used for the

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tilapias. In this case, the tilapias of tank A were used as control and fed once a day with pellets of commercial food sold by local market. The tilapias of tank B were fed once a day with commercial food plus either Ni^{2+} or Al^{3+} at concentration of 50 mg of metal per kg of food from 1 to 12 months. Thermostatic devices with temperature controllers (Conthermer Heater-300 V, Otto) were used to keep the temperature at 21 ± 3°C, at the beginning of the winter season.

Preparation of tilapia sample

Tilapia samples, including viscera, heads, and muscle tissues, were isolated and dried in a heater at 100°C. The dry material was crushed in a ceramic plate. Sample amounts of 5.0000 ± 0.0001 g of feces, 3.0000 ± 0.0001 g of muscle tissues, and 2.0000 ± 0.0001 g of either fish viscera or heads were used in chemical analysis. Feces, viscera, heads, and muscle tissues were digested in concentrated nitric–perchloric acid (20 mL HNO_3/5 mL $HClO_4$). The mixtures were concentrated in an Erlenmeyer flask by heating to 150°C and the final volume (about 10 mL) was transferred to a 25-mL volumetric flask. The samples were diluted with deionized water. In this methodology, the organic matter was completely oxidized.[1]

Preparation of samples for SWV analysis of Ni^{2+} and Al^{3+}

Fe^{3+}, Zn^{2+}, and Cu^{2+}, other metallic ions found in tilapia samples, do not interfere with the determination of Ni^{2+}. Thus, the tilapia samples prepared were used without previous treatment. Fe^{3+} and Al^{3+} were removed from tilapia samples by the following chemical method with some modifications: A NH_4Cl + NH_4OH buffer solution was added with a sample aliquot until the complete precipitation of $Al(OH)_3$ and $Fe(OH)_3$. Then, after adding 3.0 mol L^{-1} NaOH plus 3% H_2O_2 (v/v) dropwise, the solution was heated in a thermostatic bath until the dissolution of the hydroxides and the oxidation of Al^{3+} to aluminate (AlO_2^-). After centrifugation and filtration of $Fe(OH)_3$ with a 0.45-μm Millipore membrane, drops of HNO_3 were added to the aluminate solution until the complete recovery of Al^{3+}.[12]

Work electrode preparation

The electrochemical cell contents included the glassy carbon microelectrode as work electrode, platinum electrode as counter electrode, Ag/AgCl as reference electrode and 0.1 mol L^{-1} mercury nitrate [$Hg(NO_3)_2$] as supporting electrolyte. The working glassy carbon microelectrode with a mercury thin film was prepared by scanning the cyclic voltammetry potential with initial potential of 0 and vertex potential of –1.5 V *versus* the reference electrode of Ag/AgCl. An anodic peak corresponding to the oxidation of Hg to Hg^{2+} was observed at +0.8 V during the application of a potential from –1.5 to +1.5 V. The measurements were taken on Autolab GPES 663 VA Stand potentiostat/galvanostat.

Parameters for SWV measurements of Ni^{2+} and Al^{3+}

The experiments were carried out on an Autolab GPES 663 VA Stand potentiostat/galvanostat using a three-electrode system with a glassy carbon working microelectrode with a mercury thin film, a platinum counter electrode, and an Ag/AgCl reference electrode. The pulse parameters used in the SWV measurements of both metals were potential step of 20 mV, pulse height of 25 mV, and frequency of 50 Hz. SWV was used after bubbling pure N_2 through the cell content of 20 mL of a specific supporting electrolyte. To determine the amount of the DMG-Ni complex, we used 0.1 mol L^{-1} sodium sulfite solution with pH 9.6, and 0.1 mol L^{-1} ammonium citrate solution for pH 4.2. The required volumes of either 0.1 mol L^{-1} NH_3, HCl, or NaOH solutions were used to adjust pH. The different supporting electrolytes, analyte concentrations, and pH values were investigated.

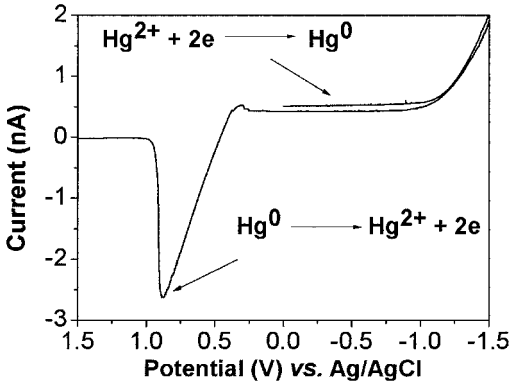

Fig. 1 Cyclic voltammogram of mercury deposition on glassy carbon electrode surface.

The voltammetric determination of free Al^{3+} ion with a mercury electrode is difficult because of its particular electrochemical behavior. Al^{3+} irreversibly reduces at –1.70 V *versus* Ag/AgCl as a reference electrode because of the hydrogen background current.[13] Al^{3+} may be determined by stripping anodic voltammetric technique as a complex with Alizarin S using a buffer solution 0.1 mol L^{-1} NH_4Cl plus NH_4OH at pH 8.2 as a supporting electrolyte. In this sense, the best deposition potential used in the analyses was –0.8 V *versus* SCE as a reference electrode and a hanging mercury drop working electrode.[14-16] In this work, 20 mL of buffer solution 0.1 mol L^{-1} NH_4Cl plus NH_4OH at pH 8.3 was transferred to the electrochemical cell content with a glassy carbon working microelectrode with a mercury thin film, platinum as a counter electrode, and Ag/AgCl as a reference electrode. After was purged the solution with pure nitrogen for 300 s, 150 μL of 4.163×10^{-4} mol L^{-1} Alizarin R was added and the solution was purged for another 30 s to obtain free Alizarin R voltammograms at approximately –0.35 V. Soon afterwards, an amount of 100 μL of Al^{3+} sample was added and the voltammograms of the Alizarin R-Al complex were observed at approximately –0.5 V. The quantitative determination of Al^{3+} was carried out by excess addition of standard Alizarin R to the cell. Deionized water was used in the voltammetric analysis. All PTFE and polypropylene containers and vessels were soaked in about 3.0 mol L^{-1} HNO_3 solution for 24 h and rinsed with deionized water before use to prevent contamination by Al^{3+}. The detection limits of Ni^{2+} and Al^{3+} by using DMG and Alizarin R as complexing agents were estimated by the lower signal obtained in the sequential addition of standard metallic solutions under supporting electrolyte. Sensitivity was not assessed; however, it can be considered lower (2 to 3 times) than the detection limits obtained.

Results and Discussion

Work electrode preparation

Figure 1 shows a cyclic voltammogram of Hg deposition on the glassy carbon microelectrode surface. A solution of 0.1 mol L^{-1} [$Hg(NO_3)_2$] was used in the voltammogram to reduce the Hg^{2+} solution on the electrode surface to metallic Hg^0. A potential from 0 to –1.5 V *versus* the reference electrode (Ag/AgCl) was applied. Then, potentials from –1.5 to +1.5 V were applied

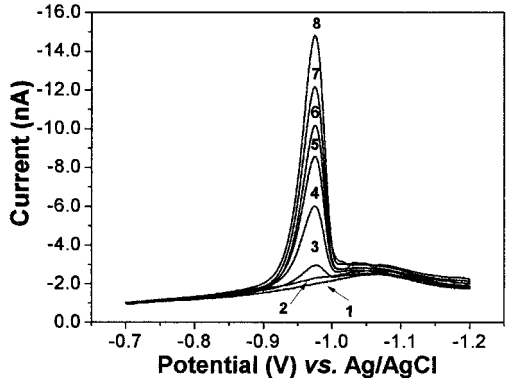

Fig. 2 Ni-DMG complex: (1) 20 mL supporting electrolyte (Na_2SO_3), (2) 10 mg L^{-1} DMG, (3) DMG plus 0.05 mg L^{-1} Ni^{2+}, (4) DMG plus 0.10 mg L^{-1} Ni^{2+}, (5) DMG plus 0.15 mg L^{-1} Ni^{2+}, (6) DMG plus 0.20 mg L^{-1} Ni^{2+}, (7) DMG plus 0.25 mg L^{-1} Ni^{2+}, (8) DMG plus 0.30 mg L^{-1} Ni.

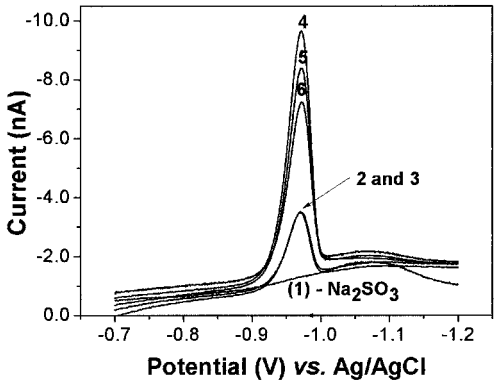

Fig. 3 Variation of the deposition potential applied: (1) supporting electrolyte Na_2SO_3, (2) Ni-DMG, –1.2 V, (3) Ni-DMG, –1.0 V, (4) Ni-DMG, –0.8 V, (5) Ni-DMG, –0.6 V, (6) Ni-DMG, –0.4 V.

to prove that Hg was deposited on the glassy carbon microelectrode surface. The potential of +0.8 V showed that the metallic Hg^0 on the surface of the glassy carbon microelectrode was oxidized to Hg^{2+} and dissolved in the solution.

Parameters for the determination of Ni^{2+} by SWV

Quantification of metals by voltammetric methods may be affected by the properties of the supporting electrolyte solutions. Four electrolytic solutions: 0.1 mol L^{-1} sodium sulfite, pH 9.6; 0.1 mol L^{-1} ammonium citrate, either pH 9.6 or 5.8 or 4.2; and buffer solution, 0.1 mol L^{-1} ammonium chloride plus NaOH, pH 8.2, were tested to find the best conditions for the determination of Ni^{2+} using DMG as a complexing agent. The best electrolytic solution for the analysis of Ni^{2+} using DMG as a complexing agent was sodium sulfite at pH 9.6. Sodium sulfite was also analyzed at pH 7.6, 8.6, and 10.6; however, no peak was observed for the Ni-DMG complex. Ammonium citrate at either pH 9.6, 5.8, or 4.2 could not be used because its high ionic mobility interferes with the determination of Ni^{2+} from the Ni-DMG complex.[17] Unfortunately, voltammetry is very sensitive to interferences by dissolved organic matter in the sample solution, which often limits its suitability, principally when the supporting electrolyte has high ionic mobility.[17] It was not possible to use 0.1 mol L^{-1} ammonium chloride plus NaOH at pH 8.2 buffer solution because the free Ni^{2+} peak at –0.85 V interferes with the determination of the Ni-DMG complex.

Figure 2 shows the analytical curve for the determination Ni^{2+} by SWV with DMG as a complexing agent. The results show that the Ni-DMG complex stripping peak current has a linear relationship in the concentration range from 1.70×10^{-7} to 5.45×10^{-6} mol L^{-1} with 0.1 mol L^{-1} sodium sulfite as a supporting electrolyte at pH 9.6. The linear correlation coefficients were close to 0.99. These results indicate that the quantitative determination of the Ni-DMG complex using sodium sulfite as a supporting electrolyte has excellent reproducibility. The best conditions for the determination of Ni^{2+} were 0.1 mol L^{-1} Na_2SO_3 (sodium sulfite) solution as a supporting electrolyte at pH 9.6 and a Ni-DMG complex concentration range from 1.70×10^{-7} to 5.45×10^{-6} mol L^{-1} as confidence limit. Other metals besides Ni^{2+} were identified in tilapia samples; however, they did not interfere with the voltammetric analysis of Ni^{2+}. Figure 3 shows the best deposition potential for the determination of Ni^{2+} by using DMG as a complexing agent. The signal of the Ni-DMG complex increased in the potential range from –0.4 to –0.8 V and decreased from –1.0 to –1.2 V. The best deposition potential was found to be –0.8 V; however, all the experiments were carried out at –0.6 V. The Ni-DMG complex peak increased and was definitely better after 30-s deposition time. Other parameters used to determine Ni^{2+} in biological samples (tilapia, *Oreochromis niloticus*) were 90-s deposition time and 0.1 mol L^{-1} sodium sulfite as a supporting electrolyte.

Parameters for the determination of Al^{3+} by SWV

The best determination conditions for Al^{3+} by SWV were selected using a three-electrode system with a glassy carbon working microelectrode with a mercury thin film, a platinum counter electrode, and an Ag/AgCl reference electrode. The best purging time obtained while bubbling pure nitrogen through the solution under stirring was 300 s. The current rate selected for the experimental data ranged from 10 nA to 100 μA with a scan rate of 50 mV s^{-1}, pulse amplitude of 100 mV, and equilibrium time of 15 s. The voltammetric anodic curves were investigated in the potential range from –0.8 to –0.05 V. Figures 4a - 4c show the best deposition potential, scan rate, and deposition time, respectively, for the formation of the Alizarin R-Al complex. The deposition potential found was –0.8 V, which supports a large formation of the complex. In addition, the best scan rate and deposition time obtained for the formation of the Alizarin R-Al complex were 50 mV s^{-1} and 60 s, respectively. The best SWV analysis conditions to determine Al^{3+} were: accumulation time of 60 s (depending on the amount of Al^{3+}), anodic potential of –0.5 V, deposition potential of –0.8 V, scan rate of 50 mV s^{-1} and pulse amplitude of 100 mV. The voltammetric anodic curves were recorded over the potential range from –0.8 to –0.05 V. The detection limit found for Alizarin R-Al complex was 1.0×10^{-8} mol L^{-1}.

Determination of Ni^{2+} and Al^{3+} in tilapia samples by SWV

Figure 5 shows the standard addition of Ni^{2+} under supporting electrolyte with tilapia sample plus DMG. The complex peak was observed at anodic potential of –0.95 V, while free DMG was determined at –0.9 V. However, DMG concentrations above 0.025 mg L^{-1} may interfere with the complex formation due to the overlapping anodic peaks of the Ni-DMG complex and free DMG. The free Ni^{2+} peak may be observed at anodic potential of –0.8 V. Nevertheless, in this study, it was not observed because the Ni^{2+} added to the cell was used in the complex formation in excess DMG. As the limit of confidence for the determination of Ni^{2+} in tilapia samples ranged from 1.70×10^{-7} to 5.45×10^{-6} mol L^{-1}, samples with concentrations above

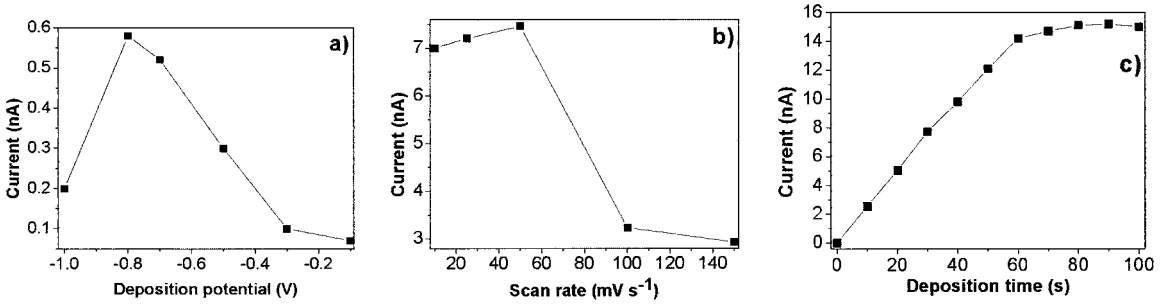

Fig. 4 Deposition potential for the determination of Al^{3+} as an Alizarin R-Al complex (a), scan rate (b), deposition time (c). Parameters: Al^{3+}, 1.81×10^{-5} mol L^{-1}; Alizarin, 2.16×10^{-6} mol L^{-1}; supporting electrolyte, NH_4OH plus NH_4Cl 0.1 mol L^{-1} at pH 8.2; scan rate, 50 mV s^{-1}; amplitude, 100 mV.

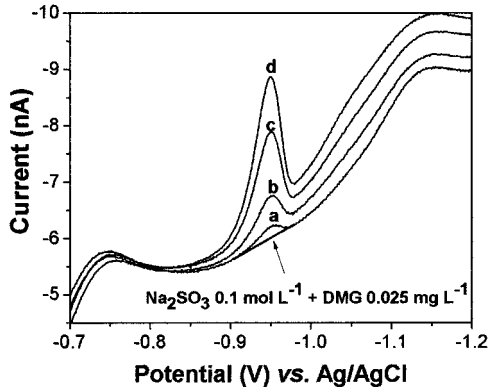

Fig. 5 Determination of Ni^{2+} in tilapia head using 0.1 mol L^{-1} Na_2SO_3 as a supporting electrolyte at pH 9.6. (a) Fish sample, (b) fish sample plus 100 μL of 100 mg L^{-1} Ni^{2+}, (c) fish sample plus 200 μL of 100 mg L^{-1} Ni^{2+}, (d) fish sample plus 300 μL of 100 mg L^{-1} Ni^{2+}.

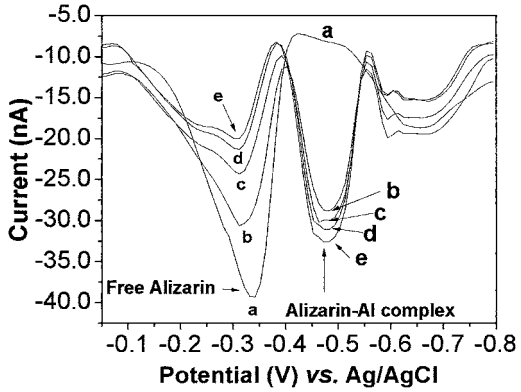

Fig. 6 Determination of Al^{3+} in tilapia head. (a) 20.00 mL of buffer solution 0.1 mol L^{-1} NH_3/NH_4Cl at pH 8.3 as a supporting electrolyte plus 150 μL of Alizarin solution with final concentration in cell of 2.16×10^{-6} mol L^{-1}, (b) combined with 100 μL of tilapia sample, (c), (d), and (e) combined with 20, 30, and 40 μL of 3.73×10^{-3} mol L^{-1} Al^{3+}, respectively.

5.45×10^{-6} mol L^{-1} had to be diluted for analysis. Ni^{2+} traces in tilapia samples may be determined by SWV with DMG as a complexing agent without interference of other metals such as Fe^{3+}, Zn^{2+}, or Cu^{2+}. In recent years, various methods have been developed to determine low concentrations of Ni^{2+} in actual samples. A voltammetric method with cation exchanger-modified carbon paste electrode was used to determine Ni^{2+}, reaching the detection limit of 8.6×10^{-8} mol L^{-1}. Dowex 50 W X 12 ion exchanger for a Nujol-graphite paste base was used as a complexing agent for Ni^{2+}.[4] SWV on a rotating-disk bismuth-film electrode allowed the simultaneous voltammetric analysis of Ni^{2+} and Co^{2+}. The detection limit found by this procedure with DMG as a complexing agent was 1.70×10^{-7} mol L^{-1}.[18] *In situ*-plated lead film electrode was proposed[19] for the simultaneous determination of nickel and cobalt by adsorptive stripping voltammetric in the presence of dimethylglyoxime (DMG) as a complexing agent. The sensitivity of this technique ranged from 5×10^{-9} to 1×10^{-7} mol L^{-1}. This method was applied to determination of Ni^{2+} and Co^{2+} in certified reference materials.[19] The animals presented morphological and hepatic changes after exposure to Ni^{2+}.[20] Ni^{2+} provokes more relevant morphological alterations in organs than other heavy metals do. The determination of trace Ni^{2+} by voltammetric methods is important because, like other heavy metals, Ni^{2+} may accumulate in organs and alter the natural structures of living being. SWV revealed viscera poisoning with 670 mg kg^{-1} Ni^{2+} in tilapias.

Figure 6 shows the Alizarin R-Al complex peak at the oxidation potential of –0.5 V. The detection limit found for Alizarin R-Al complex was 1.0×10^{-8} mol L^{-1}. The results in this figure were obtained for the following cell components: supporting electrolyte, Alizarin R, tilapia samples, and Al^{3+} standard. The solution was purged for 300 s in the current range from 10 nA to 100 μA, equilibrium time of 15 s, deposition potential of –0.8 V, scan rate of 50 mV s^{-1}, deposition time of 60 s, and amplitude of 100 mV. The concentrations of Al^{3+} were determined by the standard addition method.[21] As shown in Fig. 6, peak (a) is the signal of free Alizarin R. Peaks (b, c, d, and e) are representative of the formation of the Alizarin R-Al complex. The amounts of Al^{3+} per kg found in samples of tilapia raised in cemented tanks were: muscle tissue, 34.9; viscera, 88.2; head without gills, 126.9 mg. Other metals, such as Cu^{2+} and Zn^{2+}, did not interfere with the determination of Al^{3+} by SWV or have any observable effect on fish. In contrast, Fe^{3+} was a major interferant in the determination of Al^{3+}, requiring its removal from samples. In contrast to other methods, the method developed in this work is viable and may be applied to the determination of trace Al^{3+}. Trace Al^{3+} in food and water has been determined by Al^{3+}-cupferron complex in 0.4 mol L^{-1} $(NH_4)SO_4$ solution as a supporting electrolyte with hanging mercury drop electrode with detection limit of 8×10^{-10} g mL^{-1}.[22] In addition, the amounts of trace Al^{3+} were obtained applying scanning potential in the cathodic direction by using Pyrogallol Red and tetrabutylammonium tetrafluoroborate. In

this case, the detection limit found was of 4.8×10^{-9} mol L^{-1}.[23] The determination of trace Al^{3+} and Ni^{2+} is very important because in our previous work we detected that these metals may be toxic to living beings. Nevertheless, they must be determined quantitatively.[24-27] In the last years, we have studied the toxic effects of various heavy metals on fish.[1] Therefore, the methods developed here have been widely applied to the determination of metals in ecotoxicological investigations conducted by Universidade Estadual de Maringá and Universidade Estadual de Campinas. The main advantages of the tested technique over others such as ICP-AES are its low cost and easy operation.

Conclusion

Metals such as Zn^{2+}, Fe^{3+}, and Cu^{2+} do not interfere with the determination of Ni^{2+} by SWV, while Fe^{3+} interferes with the determination of Al^{3+}. Ni^{2+} was quantitatively determined by SWV with DMG as a complexing agent, while Al^{3+} was determined with Alizarin R as a complexing agent. The detection limit for Ni-DMG and Alizarin R-Al complexes were 1.70×10^{-7} and 1.0×10^{-8} mol L^{-1}, respectively. The methods proposed support the determination of trace Ni^{2+} and Al^{3+} in tilapias. The procedure proposed in this work has the advantages of fast complexation between the metal and the ligant, low detection limit, instrumental simplicity, and speed of analysis.

Acknowledgements

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References

1. A. T. Paulino, J. A. A. Tessari, E. M. Nogami, E. Lenzi, and J. Nozaki, *Bull. Environ. Contam. Toxicol.*, **2005**, *75*, 42.
2. E. Merian, M. Anke, M. Ihnat, and M. Stoeppler, "*Metals and Their Compounds in the Environment: Occurrence, Analysis and Biological Relevance*", **2004**, John Wiley & Sons, New York.
3. E. F. Pane, J. G. Richards, and C. M. Wood, *Aquat. Toxicol.*, **2003**, *63*, 65.
4. P. González, V. A. Cortínez, and C. A. Fontán, *Talanta*, **2002**, *58*, 679.
5. Agency for Toxic Substances & Disease Registry, Department of Health and Human Services, *Resumen de Salud Pública*, Níquel. CAS#: 7440-02-0, August, **2005**, www.atsdr.cdc.gov/es/phs/es_phs15.pdf
6. J. P. Buchet, D. Lison, M. Ruggeri, V. Foa, G. Elia, and S. Maugeri, *Arch. Toxicol.*, **1996**, *70*, 773.
7. E. Gauthier, I. Fortier, F. Courchesne, P. Pepin, J. Mortimer, and D. Gauvreau, *Environ. Res.*, **2000**, *84*, 234.
8. M. I. Yousef, *Toxicology*, **2004**, *199*, 47.
9. C. Exley, *J. Fish Biol.*, **1996**, *48*, 706.
10. A. T. Paulino, L. B. Santos, and J. Nozaki, *Toxicol. Environ. Chem.*, **2007**, *87*, 363.
11. M. J. Shaw and P. R. Haddad, *Environ. Int.*, **2004**, *30*, 403.
12. A. O. Ohlweiler, "*Separation Methods. Analytical Chemistry Quantitative*", Livros Técnicos e Ciêntificos, Rio de Janeiro.
13. J. A. Del Pozo, A. C. Garcia, and A. Blanco, *Anal. Chim. Acta*, **1993**, *273*, 101.
14. A. J. Downard, H. K. J. Powell, and S. Xu, *Anal. Chim. Acta*, **1991**, *251*, 157.
15. J. Opydo, *Talanta*, **1997**, *44*, 1081.
16. J. Di, S. Bi, T. Yang, and M. Zhang, *Sens. Actuators*, **2004**, *99*, 468.
17. A. F. Othman, *Microchem. J.*, **2003**, *75*, 119.
18. M. Morfobos, A. Economou, and A. Voulgaropoulos, *Anal. Chim. Acta*, **2004**, *519*, 57.
19. M. Korolczuk, K. Tyszczyk, and M. Grabarxzyk, *Electrochem. Commun.*, **2005**, *7*, 1185.
20. S. Morais and M. C. Pereira, *J. Trace. Elem. Med. Biol.*, **2000**, *14*, 48.
21. K. Julshamn, K. Andersen, Y. Willassen, and O. R. Braekkan, *Anal. Biochem.*, **1978**, *88*, 552.
22. L. Qiong, W. Lirong, X. Danli, and L. Guanghan, *Food Chem.*, **2006**, *97*, 176.
23. V. Arancibia and C. Muñoz, *Talanta*, **2007**, *73*, 546.
24. J. I. Simionato, A. T. Paulino, J. C. Garcia, and J. Nozaki, *Polym. Int.*, **2006**, *55*, 1243.
25. A. T. Paulino, F. A. S. Minasse, M. R. Guilherme, A. V. Reis, E. C. Muniz, and J. Nozaki, *J. Colloid Interface Sci.*, **2006**, *301*, 479.
26. A. T. Paulino, M. R. Guilherme, A. V. Reis, E. B. Tambourgi, J. Nozaki, and E. C. Muniz, *J. Hazard. Mater.*, **2007**, *147*, 139.
27. A. T. Paulino, L. B. Santos, and J. Nozaki, *React. Funct. Polym.*, **2008**, *68*, 634.