QUANTIFICATION OF NICKEL SPECIES IN PHYSIOLOGICAL MEDIA USING MICROELECTRODES

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ABSTRACT

Square wave voltammetry at microdisk gold electrodes modified with a mercury film was used to quantify nickel levels in kidney samples of mice previously treated with a solution containing those species. Comparison of the results obtained by electrochemical techniques and atomic absorption spectrometry indicates that microelectrodes are a reliable quantitative technique and greatly enhances the working conditions.

Keywords: Nickel; microelectrodes; square wave voltammetry

INTRODUCTION

The properties that characterize the microelectrodes such as mass transport, discrimination against charging current, decreased distortion from iR drop and its small size as well as the electrochemical techniques available [1], are a very useful instrument for use as medical devices to monitor and characterize ions released from metal biomaterials.

The biological fluids are extremely aggressive for the metallic implants, provoking degradation which results in the release of metallic ions for the tissues surrounding the implant, entering to the blood stream and partially accumulated in the blood filtration

organs such as liver, kidney and spleen [2] as well as in some reproductive organs [3]. The alloy more used for orthopedic implants is AISI 316L stainless steel. This material has a percentage of nickel 13-16 % weight by weight.

Furthermore, nickel is an alloying element in dental casting alloys, namely Rexalloy, Regalloy T and Vera Bond, in a very high percentage, 63-77 %, weight by weight [4] and this metal was identified to be a human carcinogen [5]. These dental casting alloys are characterized as active/passive alloys and rely on a protective oxide coating for corrosion resistance in the oral and tissue environment. It has been suggested that 16-27 % chromium provides an adequate protective oxide film and that molybdenum (Mo), manganese (Mn), titanium (Ti) and copper (Cu) additions will further enhance their corrosion resistance. Damage of the oxide film increase the levels of corrosion products which are released to adjacent and systemic tissues. It is already known that there is a risk of developing an allergic reaction to nickel and other alloying constituents. Concerns over the toxicity of corrosion products exist as well [5].

The aim of the present study was to characterize the nickel behaviour in biological solutions as well as quantification of nickel levels *in vivo* in mice organs such as liver and kidney after subcutaneous injections with Ni species using gold (Au) microdisk electrodes with a mercury film grown at the tip, by performing cyclic (CV) and cathodic stripping square wave (SWV) voltammetry measurements. Comparison between data acquired by these electrochemical techniques using microelectrodes and atomic absorption spectometry (AAS) is presented and discussed.

EXPERIMENTAL

In order to simulate the *in situ* degradation process, several mice were subcutaneously injected with 0.5 mL of a physiological solution, Hank's balanced Salt Solution (HBSS), containing 150 ppm of Ni ions dissolved from nickel chloride salt, at days 0, 3, 9 and 13. Control mice were only injected with the HBSS. The animals were sacrificed at days 4, 10 and 14 and the liver, kidney and spleen organs were removed for analysis. The removed organs were placed in appropriate vessels and they were dried in order to

eliminate the water content in a microwave oven model CEM MDS 2000.

After the drying stage, the organic materials were digested with nitric acid, HNO_3 suprapur, using again the microwave oven. This digestion is not capable to destroy completely the organic matter. The digestion proceeded with the evaporation of the resulting solutions with perchloric acid, $HClO_4$, to dryness. Finally the resulting residues were diluted with triply distilled water to an appropriate volume.

In the solutions to be analyzed was added ammonium buffer solution, 2.0 mol/L and a 0.1 mol/L of dimetilglyoxime (DMG), to a final concentration in solution of 0.1 mol/L and 0.1 mmol/L, respectively. The final pH was around 9.2. The experimental voltammograms were obtained using a potentiostat/galvanostat Model Autolab from ECO chemie equipped with a module ECD which was connected to a PC model 1120 SX, used in conjunction with a three-electrode electrochemical cell placed in a polarographic Stand 663 VA from Metrohm. The working electrode was a mercury-coated gold microelectrode of 25 μm diameter; an Ag/AgCl as the reference electrode and the auxiliary electrode was made of carbon. The mercury films were made by electrodeposition at 0.0 V during 60 s from a solution of 6 mmol/L $HgCl_2$, 1 mol/L potassium nitrate and 0.5% of HNO_3.

The experimental parameters used by the electrochemical techniques were: deposition potential -0.7 V; deposition time 5-30 s depending of the Ni concentration present in the solutions; cleaning potential -1.2 V; and cleaning time 30 s.

The CV measurements were conducted using a scan rate of 500 mV/s and all the SWV measurements were performed using a 5 mV step, an amplitude of 20 mV and a frequency of 100 Hz. Ni levels in the solutions were quantified by the standard addition method. All measurements for the same sample were repeated four times. The atomic absorption determinations were done using a Perkin-Elmer Model 4100 ZL spectrometer.

RESULTS AND DISCUSSION

The process used for the determination of nickel in these biological solutions involves adsorption and accumulation of a complex, Ni(II)-DMG, at a mercury film microelectrode and subsequent reduction of the adsorbed Ni complex. From the cyclic voltammograms presented in Fig. 1, the cathodic peak was observed at a potential around -1.03 V and it arises from reduction of Ni complex which is absorbed on the mercury film microelectrode. The absence of a peak in the anodic branch of the cyclic voltammograms indicates that the reduction of the complex is an irreversible process. On the other hand the absence of the cathodic peak for the second scan indicates that the reduction of this complex was exhausted during the first scan.

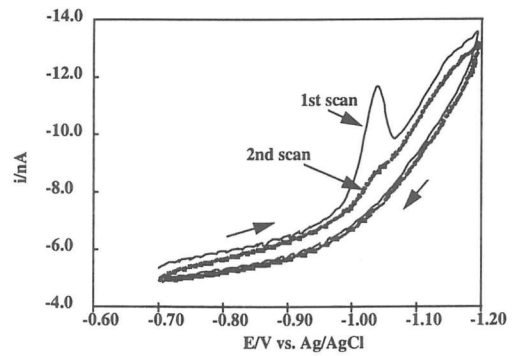

Figure 1- Cyclic voltammograms for a sample of kidney injected with Ni solution after deposition of 30 s onto microelectrode, υ= 500 mV.

In Fig. 2A it is plotted several SWV voltammograms obtained for kidney samples at 10 days, by the standard addition method which allowed to quantify the amount of this metal ion. Thus the complex peak in the original sample was quantified using the resulting standard addition plot (Fig. 2B). Because the inherent sensitivity of the method, short preconcentration times, 5-20 s, were used.

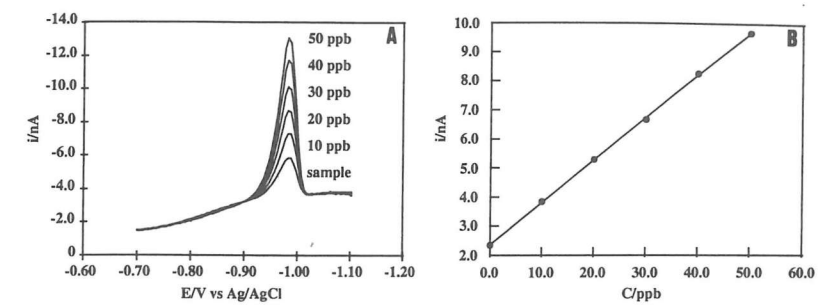

Figure 2- Determination of Ni levels in mice kidney sample at day 10: A) by SWV using the standard addition method with a deposition time of 15 s; B) Plot of the standard addition method used to quantify the Ni amounts in the sample, concentration 16.1 ppb.

Finally, in table I it is shown the results obtained for kidney mice in μg/g per dry weight, at the days of sacrifice, determined electrochemically and by AAS. As can be seen from the table the values obtained by both methods are in the same magnitude, i.e. they are in good agreement. The results obtained for kidney animals demonstrates that the nickel levels significantly increased with time when compared with the control mice, four times more at day 4 and two times more at day 10.

Table 1- Comparative results obtained in kidney mice by SWV and AAS.

		Control μg/g [a]	rsd %	4 days μg/g [a]	rsd %	10 days μg/g [a]	rds %	14 days μg/g [a]	rds %
Kidney	**SWV**	**0.368**	**2.10**	**1.59**	**2.46**	**1.35**	**1.93**	**4.92**	**2.73**
	AAS	**0.355**	**2.47**	**1.55**	**2.39**	**1.31**	**2.75**	**4.76**	**4.32**

[a] **Mean for four separate determinations and values attained by dry weight**

CONCLUSIONS

The SWV technique associated with a gold disc microelectrode modified with a mercury film is suitable for determining low nickel concentrations in these biological samples. Furthermore, the use of this technique when compared with the AAS, shows

that the risk of contamination was strongly reduced. The analysis time in SWV with the microelectrodes are much lower, compared with conventional mercury electrodes and AAS making this technique very attractive for its decrease of time associated with the excellent reproducibility of the method.

Moreover, it has been verified a significative increase in the nickel levels with time in kidney and liver indicating that there is an accumulation of these species in the blood filtration organs studied, contradicting some studies already realized which have reported that nickel was rapidly eliminated in the urine and that the level in the organs was similar to that of control animals [6].

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REFERENCES

1. "Microelectrodes: Theory and Applications", (Eds.) M. I. Montenegro, M. A. Queirós, J. L. Daschbach, NATO-ASI, Kluwer Academic Publishers, The Netherlands, 1991.

2. M. L. Pereira, A. Silva, R. Tracana and G. S. Carvalho, *Cytobios*, **77** (1994) 73.

3. J. P. Sousa, P. Lemos, P. Laranjo, G. S. Carvalho and M. L. Pereira, in "Compatibility of Biomedical Implants", (Eds.) P. Kovacs and N. S. Istephanous, The Electrochemical Society, Inc., Pennington, NJ, 1994, pp. 167-172.

4. J. D. Bumgarder and L. C. Lucas, in "Compatibility of Biomedical Implants", (Eds.) P. Kovacs and N. S. Istephanous, The Electrochemical Society, Inc., Pennington, NJ, 1994, pp. 241-251.

5. T. Norseth, *Ann. N. Y. Acad. Sci.*, **534** (1988) 377.

6. K. Merrit, T. D. Crowe and S.A. Brow, *J. Biomed. Mater. Res.*, **23**, (1989) 845.

ELECTROCHEMICAL CHARACTERISATION OF IRON SPECIES IN OSTEOBLAST-LIKE CELL CULTURES MEDIUM

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SUMMARY

Conventional gold electrode, gold, and mercury coated microelectrodes were used to study the electrochemical behaviour of iron species in osteoblast-like cell cultures medium. Gold microelectrodes, with or without surface modification, have shown to be useful tools for *in vitro* qualitative and quantitative analysis of iron ions released from stainless steel in biological systems.

Keywords: iron, gold/mercury coated microelectrode, *in vitro* bone formation.

INTRODUCTION

The biomedical applications of stainless steels in bone replacement have been widely practised due to their high generalised corrosion resistance, workability conditions, good mechanical properties and relatively low price [1]. AISI 316L stainless steel is the most commonly used metallic implant in orthopaedic surgery. The biocompatibility of these metallic implants is controlled by the chemical, or more precisely the electrochemical interaction that results in the release of metal ions into the tissue, and the toxicology of these released debris [2]. The main goal of our research is to determine the relationship between stainless steel corrosion products and its separate components on *in vitro* bone formation. In the present study only the behaviour of iron species released from AISI 316L stainless steel was investigated using a conventional gold electrode, gold, and mercury microelectrodes.

MATERIALS AND METHODS

Metallic solutions

Type AISI 316L stainless steel (Fe 63.9%, Cr 18.0%, Ni 12.5%, Mo 2.8%, Si 1.2%, Mn 1.6% and C 0.025%, weight for weight) was anodically dissolved in Hank's Balanced Salt Solution (HBSS), which simulates the composition of physiological fluids, by imposing a constant potential of *ca.* 4 V for five hours. The resulting concentrations of the major metal ions in the slurry were determined by atomic absorption spectrometry (AAS): 566.8 μg/mL of Fe, 138.6 μg/mL of Cr and 114.7 μg/mL of Ni. The other elements were not analysed.
A salt solution of $FeCl_3.6H_2O$ containing 500 μg/mL of iron was prepared separately in HBSS.