OPTIMISATION STUDY FOR NICKEL QUANTIFICATION IN CELL CULTURE MEDIUM USING MERCURY FILM MICROELECTRODES

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SUMMARY

A procedure for quantification of nickel in cell culture medium using a mercury film microelectrode and dimethylglioxime (DMG) as the complexing ligand was developed. The results obtained by adsorptive cathodic stripping voltammetry (AdsCSV) were compared to those attained by atomic absorption spectrometry and good agreement was found.
Keywords: mercury film microelectrode, nickel, cell culture medium.

INTRODUCTION

Microelectrodes have shown to possess unique properties [1] which contribute to the improvement in the quality of the experimental data in the metal trace analysis either in aqueous or biological samples. They also have been used in other fields such as neurochemistry, spectroelectrochemistry, electrode reaction mechanisms, electronucleation and electrode kinetics studies [2-5]. For the majority of these studies metal wires (e.g. carbon, platinum and gold) were used as the working substrates. However, the tip modification with a more sensitive and selective material enhances the applicability of microelectrodes. Several authors have claimed that the use of mercury film electrodes is advantageous over the use of HMDE [6-11]. The surface modification of microelectrodes with a mercury film in combination with the technique of AdsCSV allows to achieve higher sensitivity and to determine Ni in the $\mu g\ L^{-1}$ range. In this study, an optimisation of voltammetric parameters as well as a digestion procedure was performed in order to develop a reliable procedure for Ni determination in cell culture medium and to test the effect of the biological matrix.

EXPERIMENTAL

The composition of the osteoblast-like cell culture medium was previously described elsewhere [12] and consists essentially in a buffered solution of pH 7.2±0.3 containing antibiotic antimycotic solution, foetal bovine serum, inorganic salts, amino acids and vitamins. Digestion of the medium samples was performed in a CEM Model MDS-2000 laboratory microwave oven using nitric acid (suprapur 65%). After the decomposition period, the solution was evaporated to dryness and the salts were dissolved in deionized and triply distilled water.
For the quantification of nickel by square-wave voltammetry coupled with a mercury film microelectrode (MFM), an Ag/AgCl/3.00 mol L^{-1} KCl reference electrode and a cylindrical carbon counter electrode, an AUTOLAB potentiostat/galvanostat Model

PSTAT 10 with an ECD Module (from Eco Chemie) controlled by a PC, through the GPES 3 software was used. The MFM was prepared as described previously [12] and both DMG (from a 0.10 mol L^{-1} solution prepared by dissolving the appropriate amount in 95% ethanol) and NH_3/NH_4Cl buffer were added to the sample to give a final concentration of $5.00x10^{-4}$ mol L^{-1} and 0.10 mol L^{-1} (pH=9.2), respectively. The preconcentration was carried out at -0.70 V and the potential applied to remove adsorbed Ni-DMG complexes from the MFM was -1.20 V. The square-wave parameters used were a frequency of 50 Hz, an amplitude of 20 mV and a staircase step of 2 mV.

RESULTS AND DISCUSSION

The nickel quantification by adsorptive stripping voltammetry using DMG as complexing ligand has been described previously by several authors [11, 13-21]. However, in order to find the optimum conditions for the determination of nickel in osteoblast-like cell culture medium, the voltammetric response and the peak current enhancement was evaluated as a function of numerous variables namely, deposition potential and time, ligand concentration, buffer concentration and pH, frequency, amplitude and step.

When analysing by electrochemical means the solution obtained after oxidative acid treatment, a peak due to the reduction of nitrobenzoic acids formed during the digestion process from two amino acids (e.g. phenylalanine and tryptophan) appeared at *ca.* -0.60 V (Fig. 1). This interference can be eliminated by evaporating to fumes with perchloric acid the digested solution [22]. However, this additional stage was not performed since it would promote an increase of the risk of contamination and of the time of sample treatment without any benefit for the Ni quantification.

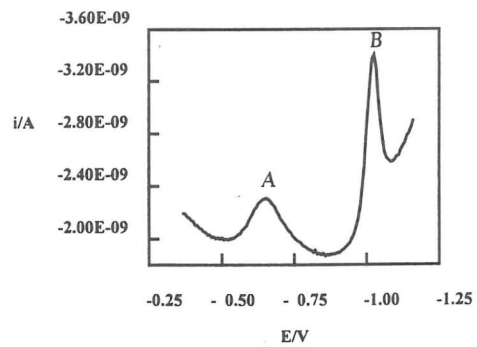

Figure 1- Adsorptive cathodic stripping voltammogram of the digested solution of cell culture medium showing (A) the interfering signal and (B) the Ni peak.

The influence of the interference signal on the reduction of Ni with DMG was studied by varying the scan rate between 50 mV/s and 7.50 V/s. Same results as those previously reported in the literature [11, 13-21] were obtained *i.e.*, total irreversibility (a linear relation was obtained between the peak potential and the logarithm of the scan rate, r=0.999) and adsorption of the reactants at the MFM (the peak current was linearly

dependent on the scan rate, r=0.999), indicating that the interference signal did not affect the reduction mechanism of Ni in the presence of DMG.

The effect of the deposition potential on the Ni peak height was evaluated by ranging the potential from -0.50 V to -0.90 V and it can be observed in Fig. 2 that the current increased sharply to *ca.* -0.72 V and then, remained approximately constant. A similar profile was observed by Economou et al. [13]. The values of the collection potential and the potential which removed the Ni-DMG complexes adsorbed at the MFM were selected as -0.70 V and -1.20 V, respectively.

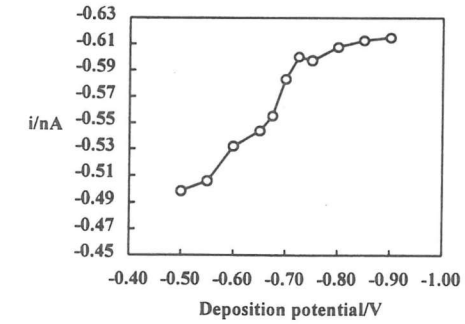

Figure 2- Effect of the adsorption potential on the Ni peak current. Conditions: deposition time 2 s; NH_3/NH_4Cl=0.10 mol L^{-1} (pH=9.2); Ni=68 μg L^{-1}; frequency=50 Hz; amplitude=20 mV and step=2 mV.

The variation of the deposition time had different effects in the Ni and interference peaks. A three time rise of the metal signal was observed by varying the time from 2 s to 300 s, in contrast with the considered insignificant 9.5% increase of the interference peak. In all analyses made, the maximum adsorption time used was 30 s because for higher times the gain in sensitivity achieved was not worthwhile when compared with the longer analysis time.

The profile attained for the effect of the complexing agent on the Ni peak was similar as the one reported in the literature [20]. Maximum value for nickel current and minimum for the interference signal were obtained for a DMG concentration of $1.00x10^{-4}$ mol L^{-1} and $5.00x10^{-4}$ mol L^{-1}, respectively. Obviously, a DMG concentration of $5.00x10^{-4}$ mol L^{-1} was selected.

The buffer concentration and pH were the main factors influencing the peak current, the stability of the complexes and their adsorption on the MFM surface. Similar results as those already described [23] were obtained and a choice of 0.10 mol L^{-1} for the NH_3/NH_4Cl concentration appeared to be the best compromise since at this value the nickel peak current was almost unaffected by variations of pH between *ca.* 9.2 and 9.3.

The square-wave parameters were also investigated (Fig.3-5) and a frequency of 50 Hz, a step of 2 mV and an amplitude of 20 mV were considered as the most reliable values bearing in mind the resolution, the baseline, peak shape and sensitivity.

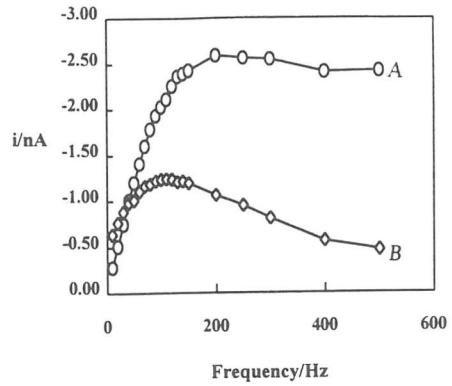

Figure 3- Dependence of the peak current of Ni (A) and of the interfering peak (B) on frequency. Conditions: deposition time=30 s; deposition potential=-0.70 V; DMG=5.00x10^{-4} mol L^{-1}; NH_3/NH_4Cl=0.10 mol L^{-1} (pH=9.2); Ni=27.3 μg L^{-1}; amplitude=20 mV and step=2 mV.

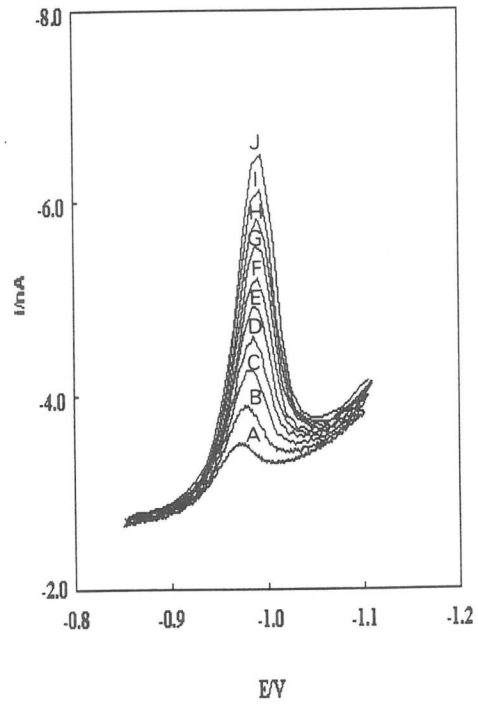

Figure 4- Voltammograms for Ni=27.3 μg L^{-1} with different steps (A) 1 mV; (B) 2 mV; (C) 3 mV; (D) 4 mV; (E) 5 mV; (F) 6 mV; (G) 7mV; (H) 8 mV; (I) 9 mV and (J) 10 mV.

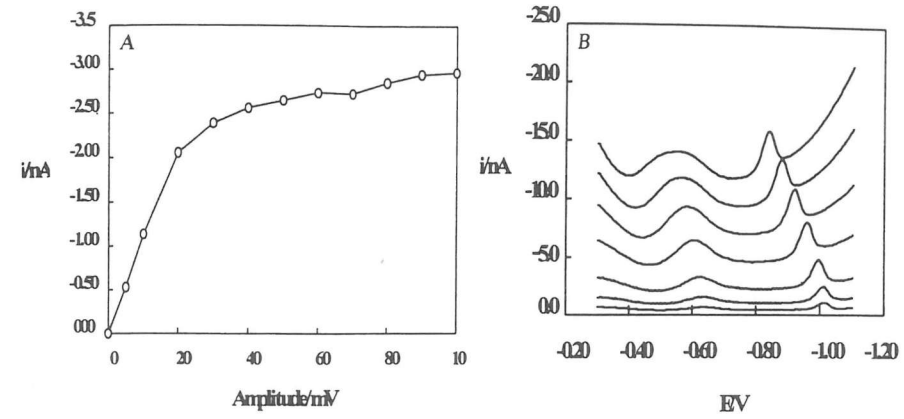

Figure 5- Effect of the amplitude on the Ni peak (A) and corresponding voltammograms with different square-wave amplitudes (B) for a solution containing 27.3 μg L^{-1}.Conditions: deposition time=30 s; deposition potential=-0.70 V; DMG=5.00x10^{-4} mol L^{-1}; NH_3/NH_4Cl=0.10 mol L^{-1} (pH=9.2); frequency=100 Hz and step=2 mV.

Fig.6 illustrates the quantification of Ni in cell culture medium by the standard addition method which eliminates the matrix effect. The accuracy of this procedure was verified by comparison of the results obtained by AdsCSV -(142±0.9) μg L^{-1}; (202±3.1) μg L^{-1}; (87.0±1.1) μg L^{-1} - with those attained by AAS - (142±0.8) μg L^{-1}; (201±0.4) μg L^{-1}; (86.3±1.3) μg L^{-1} - and good agreement was found.

The detection limit was calculated from three times the standard deviation of the blank, as recommended by IUPAC [24] and a value of *ca.* 7.70x10^{-9} mol L^{-1} was obtained for a deposition time of 10 s.

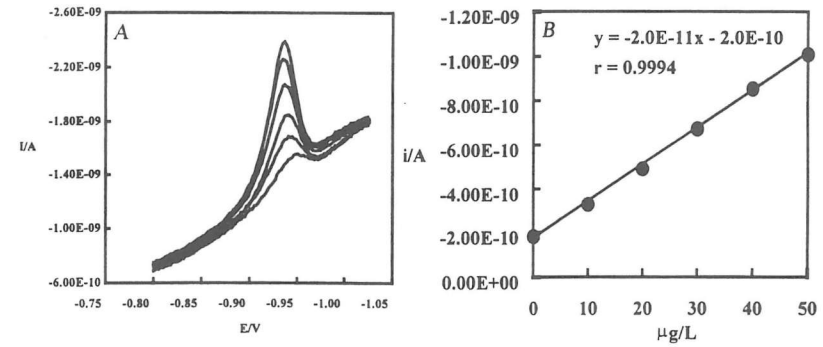

Figure 6- Voltammograms (A) and calibration graph (B) obtained by standard additions of Ni in osteoblast-like cell culture medium digest containing 10.1 μg L^{-1}of Ni. Conditions: deposition time=5 s; deposition potential=-0.70 V; DMG=5.00x10^{-4} mol L^{-1}; NH_3/NH_4Cl=0.10 mol L^{-1} (pH=9.2); frequency= 50 Hz; amplitude=20 mV and step=2 mV.

CONCLUSIONS

By optimisation of the electrochemical parameters and using the standard addition method, the presence of some organic residues in the digested samples did not affect the nickel quantification in the cell culture medium. Also, the use of a MFM coupled with square-wave voltammetry enabled us to work without removal of oxygen, without forced convection during the deposition step and without equilibration period before the scan was started.

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DETERMINATION OF TOTAL IRON IN BIOLOGICAL SAMPLE SOLUTIONS WITH MERCURY MICROELECTRODES

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ABSTRACT

The analytical conditions for the determination of the total iron in mice organ samples have been optimized and the suitable experimental parameters were found to be a catechol concentration of $3.0x10^{-4}$ mol/L, pH *ca.* 7.2 provided by PIPES buffer concentration of $8.0x10^{-3}$ mol/L, a deposition potential of -1.80 V, deposition time 10-30 s, frequency 50 Hz, step 4 mV and an amplitude of 20 mV. The detection limit was $13.7x10^{-9}$ mol/L after a deposition potential of 25 s. The relative standard deviation of fifteen repeated measurements of the same solution was 1.13%, indicating that the peak iron response was very reproducible.

INTRODUCTION

Metal ions affect the human well-being in several ways. Some of these metal ions (e.g. iron, nickel, chromium) plays an important role in life, where biological systems regulate their uptake, metabolism and excretion, consequently their concentrations in the human body are compartmentalized and well defined [1]. The presence of a metallic implant in the body such as AISI 316L stainless steel, changes its natural equilibrium because the corrosion products released from the alloy penetrate the biological tissues surrounding the implant [2] after which they enter in the blood stream and accumulates preferentially in several vital organs [3].