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].

During the last years increasing interest has been devoted to the development of electroanalytical methods to determine organic and metallic species in solution [4]. A variety of these electroanalytical methods including square wave voltammetry (SWV) have been improved [5, 6], together with the optimization of the instrumental devices and electrodes.

Mercury film microelectrodes (MFM) were reported by Anderson et. al. [7] and developed by Whigtman et. al. [8]. This devices present several advantages when compared with the classical hanging mercury dropping electrodes HMDE´s namely decrease of the deposition time, deposition under non-stirring conditions, no need for a quiescent period, less interferences by the presence of oxygen, small sample volume and much less waste of mercury.

The aim of the present study was to use the advantages of mercury film microelectrodes to quantify total iron accumulated in organs such as liver, kidney and spleen, after several injections of a prepared metallic iron solution, by performing cathodic stripping voltammetry measurements.

EXPERIMENTAL

In order to simulate the *in vivo* degradation process, several mice were subcutaneously injected with 0.5 mL of metallic iron anodically dissolved in a physiological solution (HBSS) containing 538 ppm of iron ions at days, 0, 7, 14 and 21. The control animals were injected only with HBSS. The animals were sacrificed after ether anaesthesia at a week from the day of the last injection and the liver, kidney and spleen were removed. The digestion process of these organs was described previously [9].

Voltammetric determinations were obtained using a potentiostat/galvanostat Model Autolab from Eco Chemie equipped with a module ECD to work with microelectrodes, used in conjunction with a three-electrode electrochemical cell placed in a polarographic Stand 663 VA from Metrohm. The working microelectrode of 25 µm diameter was made of a gold wire, with a mercury film grown on its surface, an Ag/AgCl as the reference electrode and the auxiliary electrode was a carbon barr. The mercury films were grown from a solution of 6 mmol/L mercury(II) chloride, $HgCl_2$ (Merck), 1.0 mmol/L potassium nitrate, KNO_3 and 0.5% of HNO_3, by electrodeposition

at 0.0 V versus Ag/AgCl during 60 s. Prior to each film the solution was dearated for 10 min with nitrogen.

The digested organ samples were pipetted into the cell. The pH was adjusted to *ca.* 7.2 by addition of PIPES buffer (piperazine-N,N´-bis(2-ethanesulfonic acid) to a final concentration of 8.0 mmol/L and the samples were purged with nitrogen during 12 min to remove oxygen. The complexing agent, catechol, was added giving a final concentration of 3.0×10^{-4} mol/L. The catechol solution was prepared daily and oxygen was removed by purging with nitrogen during 12 min. The preconcentration step was carried out at -1.80 V during 25 s for the levels of iron presented in samples. To remove the adsorbed iron complex from the mercury film a potential of -0.80 V was applied during 10 s. The square wave parameters used were a frequency of 50 Hz, an amplitude of 20 mV and a step of 4 mV.

A 1000 ppm stock solution (BDH reagents) was used daily to prepare Fe standard solutions. The atomic absorption determinations were done using a spectrometer model GBC 904AA. The Fe levels in the samples were quantified by both techniques using the standard addition method and, all measurements, for the same sample were repeated at least three times.

RESULTS AND DISCUSSION

SWV with MFM was used to determine total iron in biological sample solutions. The method involves complexation of iron with catechol, adsorption and accumulation of the complex formed onto the mercury film microelectrode and subsequent reduction of the adsorbed iron complex [10, 11].

Figure 1 shows the effect of various operational parameters on the Fe-catechol adsorptive stripping response in digested organ samples. The solution analysed was a control liver sample at day 28 with a concentration in iron of 97.2 µg/L.

The pH dependence of the peak was studied over the 6-7.4 pH range (Fig. 1a). The peak gradually increases and the maximum peak height is reached at pH 7.2. A similar pH effect was observed by van den Berg et. al. [11] in a HMDE. A negative shift in the peak potential, from -0.28 to -0.39 V was observed as the pH increases. The pH solution was adjusted with PIPES buffer. Figure 1b shows the influence of PIPES concentration on the iron peak. The peak current increases linearly from 2.0 to 8.0 mmol/L and decreases slightly for higher concentrations. The catechol concentration

also has a pronounced effect on the adsorptive response (Fig. 1c). The peak current increases rapidly with catechol concentration and the maximum value was attained at c.a. $3.0x10^{-4}$ mol/L. For higher concentrations the peak enlarges and the resolution decreases. The effect of the preconcentration step on the peak current was evaluated over the -0.10 to -1.90 V range (Fig. 1d). Maximum response is attained following a deposition potential of -1.80 V. At this potential, sometimes it is possible to observe some adsorption of Cu with catechol but this peak is very small and well separated from the iron peak (Fig. 2). Cu concentration is these biological solutions is 100-300 times less than iron and for this concentration range, Cu do not act as an interference.

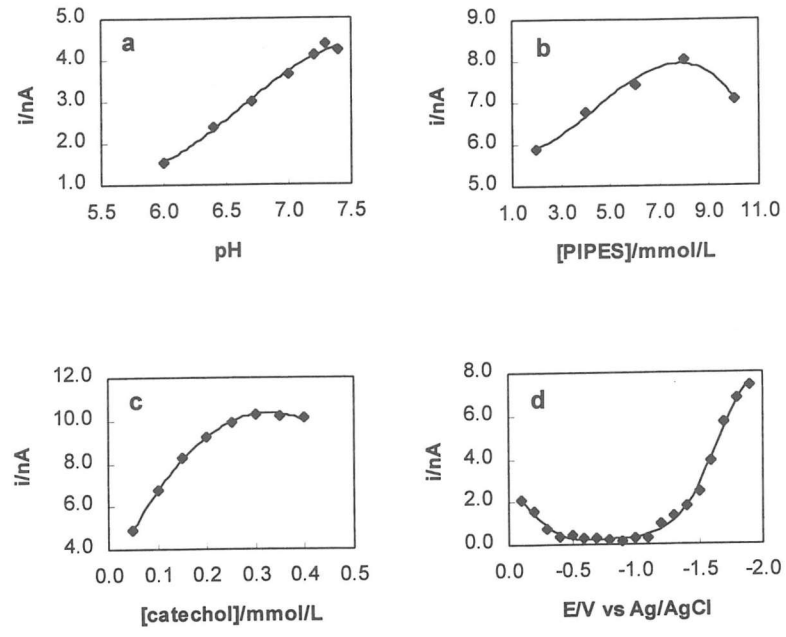

Figure 1- Effect of pH (a), PIPES concentration (b), catechol concentration (c), and deposition potential (d) on the complex Fe-catechol SWV response in a digested control liver sample at day 28 which has a Fe concentration of 97.2 μg/L.

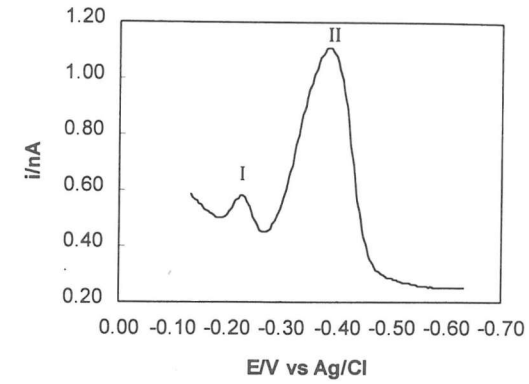

Figure 2- Square wave voltammogram of a spleen sample at day 21 containing 89.4 μg/L of Fe and 0.56 μg/L of Cu. I) Cu peak; II) Fe peak. Operating conditions: deposition potential -1.80 V; deposition time 25 s; step 4 mV; amplitude 20 mV; frequency 50 Hz.

The SWV parameters that gave the best compromise between resolution and sensitivity were achieved with a frequency of 50 Hz, an amplitude of 20 mV and a step of 4 mV.

The deposition time was changed between 5 and 70 s. The iron peak current increases linearly until 45 s (r=0.999), and for deposition times higher than 60 s the MFM was saturated for a Fe concentration of 93.6 μg/L in a control liver mice at day 7.

The range of linearity between the peak current and Fe concentration was investigated by analyzing a solution of control kidney at day 7. No deviation from linearity (r=0.999) was obtained in the range investigated, i.e. 20-200 μg/L. At concentrations above 225 μg/L deviation from linearity began, owing to surface saturation. The linear range was extended by decreasing the deposition time.

The reproducibility of the method was estimated by fifteen measurements of a control liver sample at day 28 and the relative standard deviation was found to be 1.13%.

The detection limit for the technique was 13.7 nmol/L (calculated from 3 times the standard deviation of the blank) for a deposition time of 25 s in a diluted control kidney sample at day 7.

To check the accuracy of this electrochemical process the iron concentration was

determined by AAS in all samples. The values were very similar and differed by 0.97 to 4.1%.

CONCLUSION

The present study shows that total iron levelsl present in the organs can be determined using square wave voltammetry with the adsorption of the complex iron-catechol onto the mercury film microelectrode. Due to the enhanced mass transfer at microelectrodes, the deposition time of metal ions can be accomplished without stirring the solutions and makes this technique very fast compared with a HMDE.

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RESEARCH OF DISTRIBUTION OF MEAN CURRENT IN NICKEL HYDROXIDE POROUS ELECTRODE WHILE POLARIZING WITH ASYMMETRICAL CURRENT

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At the previous work [1] we showed that the usage of alternative asymmetrical current let the possibility to get any distribution of mean current along the depth of a porous matrix, i.e. the distribution with maximum in the centre of the electrode. This fact is very important because it is the distribution of mean current that influences greatly on the coefficient of usage of the active mass and capacity taken and given by the electrode.

In this work the feature of alternative current named above was checked up on a physical model pore for nickel hydroxide porous electrode. A model pore is a glass tube with nickel wire even covered with nickel hydroxide filled with the solution (KOH 1.2 g/cm^3 + LiOH 20 g/l) and polarized on the one side (Fig. 1). In order to learn the distribution of average current along the depth of a physical model of a pore or the same along the length of a nickel wire it was slowly and even sunk into the solution of the same composition. Constant potential on the nickel wire -10 mV against mercury oxide electrode in the same solution in the point of entrance of the nickel wire into the solution was being kept (Fig. 2). At this time in the cell the current proportional to the charge of nickel hydroxide in the point of entering the wire into the solution is flowing, and the recorder connected to the cell draws the picture of distributing of average current along the length of nickel hydroxide wire. The calculation of current distribution along the depth of a pore was fulfilled with a numerical method on the base of macrohomogeneous model of a porous electrode.