

Electroanalysis of Cu, Pb, Cd and Zn in Microalgae Culture: Matrix Effect Evaluation.

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Abstract

This work illustrates, for first time, the use of simple and low-cost electroanalytical techniques to monitoring the heavy metals in microalgae cultures. The conditions of both selected techniques for the analysis, differential pulse polarography (DPP) and anodic stripping voltammetry (ASV) were optimized, giving selective and sensitive information in the simultaneous determination of Cu, Pb, Cd and Zn in presence of *Chlorella vulgaris*. The electrochemical studies by cyclic voltammetry (CV) of the heavy metals on mercury electrode and the matrix effect evaluation are included.

1 Introduction

Environmental pollution by heavy metals has become a serious problem in the world. The heavy metals are essentially non-biodegradable; therefore they accumulate in the environment or in the body tissues of living organisms (bioaccumulation) and their concentrations increase as they pass from lower trophic levels to higher trophic levels (a phenomenon known as biomagnification). The heavy metals are classified as essential and non-essential. Essential heavy metals (Fe, Mn, Cu, Zn, and Ni) are those, which are needed by living organisms in small quantities for vital physiological and biochemical functions. Non-essential heavy metals (Cd, Pb, As, Hg, and Cr) are those, which are not needed by living organisms for any physiological and biochemical functions [1].

Decontamination of heavy metals from waste water has been a challenge for a long time. A number of methods have been developed for removal of heavy metals from industrial, but these conventional tech-

nologies are expensive. Hence, an alternative bioaccumulation on biological materials may give rise to a technically reliable and economically viable clearance process. Green plants as microalgae have an enormous ability to uptake pollutants from the environment and accomplish their detoxification by various mechanisms and its use is called phytoremediation [2, 3]. It is a novel, cost-effective, efficient, environment- and eco-friendly technology.

Current techniques capable of multielement determination, atomic emission spectrometry with inductively coupled plasma excitation (ICP-AES), X-ray fluorescence (XRF), and atomic absorption spectrometry (AAS), are very expensive, time-consuming, and often do not offer adequate sensitivity for reproducible determination at trace to ultratrace concentrations of multielements in complex matrices [4]. Voltammetric techniques such as differential pulse polarography (DPP) and anodic stripping voltammetry (ASV), require relatively inexpensive instrumental analysis methods and are capable of accurate multielement determination at trace to ultratrace levels. ASV has become accepted as one the most powerful electroanalytical tools for ultra trace element analysis of water, food and biological matrices due to its extreme sensitivity, selectivity, and capability of multielemental analysis [5-8].

The aims of this study were to develop an analytical method for determination of toxic elements Pb and Cd and essential trace elements Cu and Zn by using ASV and/or DPP mode, together with the evaluation of matrix effect on analytical signals. Using the differential pulse mode, peak potentials as E_p were -0.523, -0.327, 0.078 and -0.943 V for cadmium, lead, copper and zinc, respectively. Fresh, marine and waste water have been analysed by

standard addition methods. Then, in this work, a simple and rapid ASV/DPP method has been developed for the simultaneous determination of zinc, cadmium, lead, and copper in water samples in presence of microalgae as decontaminant media.

2 Materials and methods

2.1 Reactants

Reagents were of maximum purity available and were used without further purification. Copper, Lead, Cadmium and Zinc standard solutions (1,000.0±0.2 ppm of each element and stored at 4°C) were purchased from Fluka. The chemicals used in the preparation of the universal acetic/acetate buffer solution were purchased from Aldrich. All working solutions of heavy metals were daily prepared by dilution to required concentration using Milli-Q grade water. All reagents used in the culture media assays were provided by Aqual Gae with the following composition: NaNO₃, KH₂PO₄, MgSO₄·7H₂O, CaCl₂·2H₂O, Citrato sódico, Na₂-EDTA, Na₂MoO₄·2H₂O, MnCl₂·4H₂O, ZnCl₂, CuSO₄·5H₂O, CoCl₂·6H₂O, boron, SeO₂, and vitamins (biotine or B8, cyanocobalamine or B12 and thiamine or B1).

2.2 Voltammetric analysis

Electrochemical measurements were obtained with a potentiostat μAUTOLAB Type II, connected to the 663 VA Stand (Metrohm), through IME 663, where the electrochemical cell is placed, in conjunction with the three electrode systems described below. The system was controlled by General Purpose Electrochemical experiments software 4.9 (GPES 4.9). The cell configuration consists of working electrode: mercury (SMDE mode), a reference electrode of Ag/AgCl in KCl 3M, and Pt or carbon as counter electrode. The volume dispense in is 25 mL. All potentials given hereafter will be relative to

above mentioned reference electrode. The voltammetric measurements were carried out under room temperature.

The CVs were usually recorded in the potential range from 0.2 to -1.2 V, that is, ΔU = 1.4 V, with a scan rate of 100 mVs⁻¹ and a step potential of 5 mV, unless otherwise indicated. When using DPP, the conditions were as follows: step potential of 5 mV; pulse amplitude, ΔE, of -50 mV; and scan rate of 5mVs⁻¹. When using ASV, a deposition potential and time were selected, being -1.2 V and from 60 to 600s, respectively; then, the potential was scanned to anodic direction, usually from -1.2 to 0.2 V. All electrochemical measurements were carried out in quiescent solutions and in absence of oxygen, purging 15 min with nitrogen.

3 Results and discussion

3.1 Microalgae culture characterization

The characterization of microalgae culture suspensions by control of Zeta potential (z), pH and conductivity in several photobioreactors were investigated (Table 1). It can be read that the *Chlorella vulgaris* are negatively charged and the charge is independent of the pH of the culture (as long as the values are between 6.5 and 9.0) and the conductivity (mostly due to the nutrients used as food for microalgae). These conditions are favorable for a good stability of culture suspensions, with no aggregation and coprecipitation of microalgae [9].

3.2 Voltammetric analysis

The studies by CV (Fig.1) were carried out on HMDE as working electrode in 0.8 mM acetic/acetate buffer solution (pH 5). The Fig. 1A displays the CVs of the quaternary mixture of the four ions, Cu²⁺, Pb²⁺, Cd²⁺ and Zn²⁺. It can be seen that it is easy to locate the reduction peaks of each metal when the potential was scanned from 0.2V to -1.2V at 100mV/s and also the four oxidation peaks at

Table 1: Microalgae culture characterization.

Microalgae Culture	Zeta Potential (mV)	pH (T)	Conductivity (μS/cm) (T)
PTBR-5 (1:4)	-22.2 ± 0.7	8.29 ± 0.02 (23.0°C)	475 ± 2 (22.5°C)
PTBR-6 (no dilution)	-19.1 ± 2.2	8.40 ± 0.02 (22.3°C)	543 ± 2 (22.6°C)
PTBR-7 (no dilution)	-19.3± 0.3	7.75 ± 0.02 (22.8°C)	348 ± 3 (22.4°C)
PTBR-7* (1:3)	-20.3± 2.2	6.95 ± 0.02 (25.9°C)	406 ± 3 (25.5°C)

PTBR-X –Photobioreactor (40L); * Stationary microalgae culture

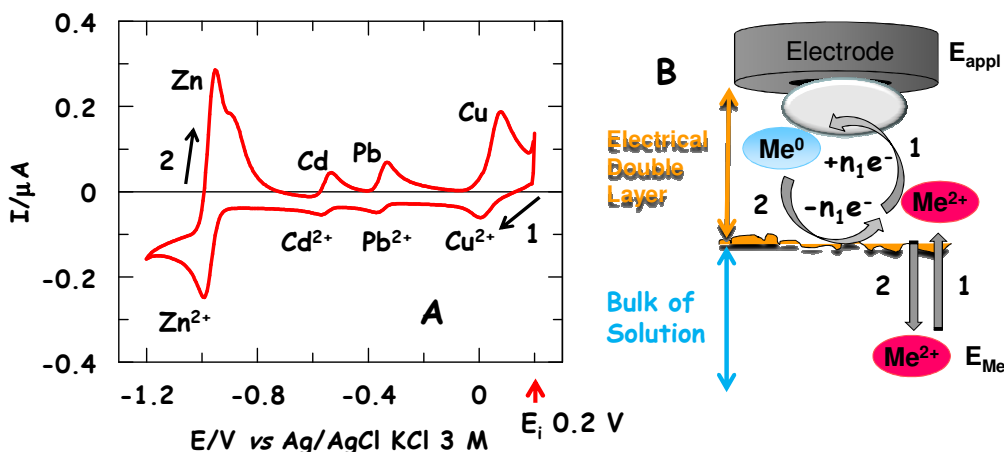


Figure 1: A.- Cyclic voltammograms of a quaternary sample with: 1.6ppm of Pb and Cu , 0.80ppm of Cd and 5.0 ppm of Zn at 100mV/s. B.- Mechanism proposed for the electrochemical process of heavy metals.

-0.952V, -0.532V, -0.332V and 0.073V that appear for the oxidation of Zn, Cd, Pb and Cu at the reverse scan. The CV voltammograms display a marked improvement in the anodic peak current, with an anodic and cathodic ratio > 1 . It also shows the formal potential (E^0) at 0.034V for Cu, -0.349V for Pb, -0.552V for Cd and -0.974V for Zn, with a peak-to-peak separation (ΔE_p) of 59mV, 34mV, 37mV and 39mV, respectively. The cations are reduced to the metal, forming an amalgam with mercury electrode, where they are preconcentrated (Fig. 1B, inset 1). The deposited metal is then oxidized at the reverse scan (Fig. 1B, inset 2). The results revealed that a quasireversible two electrons mechanism is involved in these processes. As scan rate increase from 10mV/s to 500mV/s at fixed concentration of heavy metals (results not shown), the reduction peak current of each is proportional to the square root of scan rate that is expected for reactions that involve mass transport from bulk of solution to the double layer (Fig. 1B, inset 1) and it is totally diffusion controlled.

3.3 Matrix effect evaluation and analysis

The value of the slope in the calibration curve is the direct measurement to know the sensitivity of the method. Table 2 showed that the slope of calibration curves for each metal by DPP are very dependent of electrolytic media, decreasing the slope when the sample is going more complex, indicating the matrix effect. To minimize this effect, standard addition on the water samples will be applied for the simultaneous determination of heavy metals. In all water samples analyzed (natural, tap, marine and waste water) by DPP and/or ASV, the content in Cu, Cd, Pb and Zn is 10-100 times below the limits

Table 2: Matrix effect evaluation by DPP

Metal	Slope (nA/ppm)	Slope(nA/ppm)
	Pure water + Culture media	Pure water + Culture media + Microalgae
Cu	64.00 ± 0.47	48.21±1.29
Pb	24.52 ± 1.46	16.03±0.40
Cd	42.75 ± 0.40	39.27±0.98
Zn	68.48 ± 0.83	67.15±1.51

regulated by different organisms (EPA, EEU, WHO) [10].

3.4 ASV analysis in microalgae culture

To obtain a much more sensitive peak current, ASV was applied. Fig. 2 illustrates the DP voltammograms for quaternary mixture of the four heavy metals in microalgae culture at the optimized instrumental conditions with the increasing the concentration of the analytes.

It can be seeing that the voltammetric peak of Cu overlapping with both residual current (red line, inset Fig.2) and microalgae residual current (blue line, inset Fig.2). However, it is easy to locate all oxidation peaks of each stripped metal, doing the analysis very selective.

In order to obtain the concentration range in which the response of the electrode is linear with the concentration and the detection and quantification limits, the effect of concentration of each metal on its peak currents was investigated (illustrated in same Fig.2). The calibration curve exhibits a linear relation between the peak current of Zn (-0.980 V), Cd (-0.575 V), Pb (-0.380 V) and Cu (0.031 V) and its concentration over the range 0.2-1.6 ppm for Cu and Pb ($r=0.995$), 0.04-0.8 ppm for Cd ($r=0.9999$) and 1-

5 ppm for Zn ($r=0.998$). Statistical analysis gave the following equations: $i_{pa} (\mu A) = (0.0677 \pm 0.0320) + (2.4016 \pm 0.1342) [Cu] (\text{ppm})$ ($R^2 = 0.995$); $i_{pa} (\mu A) = (0.0821 \pm 0.0306) + (0.9802 \pm 0.0311) [Pb] (\text{ppm})$ ($R^2 = 0.998$); $i_{pa} (\mu A) = (0.0172 \pm 0.0064) + (1.5226 \pm 0.0156) [Cd] (\text{ppm})$ ($R^2 = 0.999$) and $i_{pa} (\mu A) = (0.5925 \pm 0.0146) + (2.7062 \pm 0.0053) [Zn] (\text{ppm})$ ($R^2 = 0.998$). The detection and quantification limits, calculated as three and ten times the standard deviation of residual current and slope of calibration

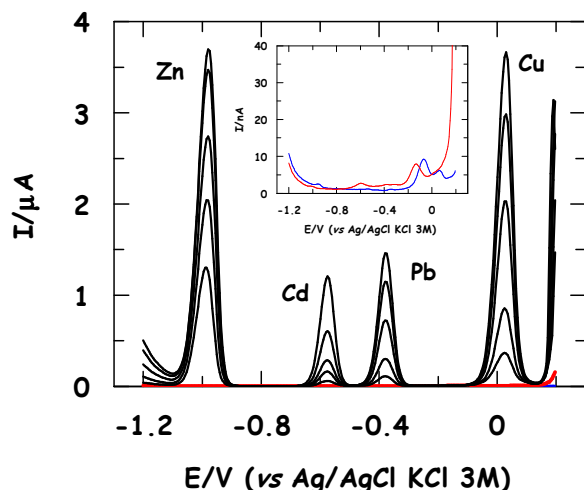


Figure 2: ASV voltammograms for quaternary mixtures of Cu, Pb, Cd and Zn at different concentrations. Conditions: $E_{dep} = -1.2V$, $t_{dep} = 60s$, $v = 5m/s$; $\Delta E = 50mV$

curve ratio, were found to be 0.165 and 0.549 ppm for Cu; 0.094 and 0.312 ppm for Pb; 0.013 and 0.400 ppm for Cd and, 0.062 and 0.207 ppm for Zn, respectively. The sensitivity can be enhanced about 6 times with the increasing of the deposition time from 60s (Fig.2) to 600s at -1.2V under stirring conditions and lower concentrations can be detected by ASV-standard addition method.

4 Conclusion

The electrochemical studies described here have shown that the method is a rapid, reproducible, and accurate determination of these elements in opaque samples can be carried out. All water samples analyzed showed matrix effect, decreasing the sensitivity in the analysis. For that, the standard addition methodology should be used to minimize this effect. Finally, the optimized voltammetric and polarographic methodology can be also used for the direct and the simultaneous analysis of these heavy metals in suspensions without a previous separation of microalgae.

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