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Title: Penicillamine-modified sensor for the voltammetric determination of Cd(II) and Pb(II) ions in natural samples

Article Type: Research Paper

Keywords: chelating agent-modified sensor; electrochemical grafting; metal determination; stripping voltammetry; D-penicillamine

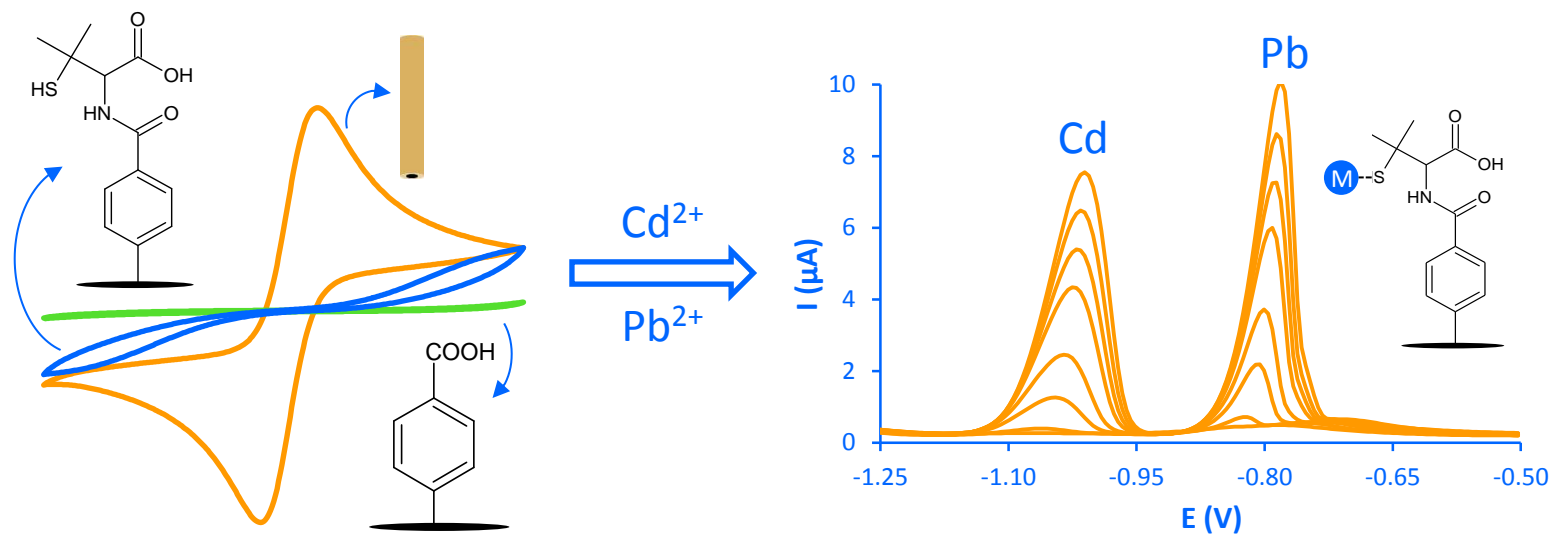
Corresponding Author: Dr Nuria Serrano,

Corresponding Author's Institution: University of Barcelona

First Author: Clara Pérez-Ràfols

Order of Authors: Clara Pérez-Ràfols; Nuria Serrano; José Manuel Díaz-Cruz; Cristina Ariño; Miquel Esteban

Abstract: A new penicillamine-GCE was developed based on the immobilization of D-penicillamine on aryl diazonium salt monolayers anchored to the glassy carbon electrode (GCE) surface and it was applied for the first time to the simultaneous determination of Cd(II) and Pb(II) ions by stripping voltammetric techniques. The detection and quantification limits at levels of $\mu\text{g L}^{-1}$ suggest that the penicillamine-GCE could be fully suitable for the determination of the considered ions in natural samples.



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4 **Penicillamine-modified sensor for the voltammetric**
5 **determination of Cd(II) and Pb(II) ions in natural samples**
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14 Clara Pérez-Ràfols, Núria Serrano*, José Manuel Díaz-Cruz, Cristina Ariño, Miquel
15 Esteban
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21 Departament de Química Analítica, Facultat de Química, Universitat de Barcelona,
22
23 Martí i Franquès 1-11, E-08028 Barcelona (Spain)
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25 Phone: 0034934039274, fax: 0034934021233, e-mail: nuria.serrano@ub.edu
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27 * to whom correspondence should be addressed
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36 **ABSTRACT**
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39 A new penicillamine-GCE was developed based on the immobilization of D-penicillamine
40 on aryl diazonium salt monolayers anchored to the glassy carbon electrode (GCE) surface
41 and it was applied for the first time to the simultaneous determination of Cd(II) and Pb(II)
42 ions by stripping voltammetric techniques. The detection and quantification limits at levels
43 of $\mu\text{g L}^{-1}$ suggest that the penicillamine-GCE could be fully suitable for the determination
44 of the considered ions in natural samples.
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56 **Keywords:** chelating agent-modified sensor; electrochemical grafting; metal
57 determination; stripping voltammetry; D-penicillamine
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1. INTRODUCTION

The determination of trace heavy metals with adverse health effects in human metabolism presents obvious concerns due to their persistence in the environment and documented potential for serious health consequences. As they cannot be degraded, heavy metals from industrial wastes, mining, lead-acid batteries, vehicle emissions, fertilizers, paints, and atmospheric depositions may enter the body through food, water, air or by absorption through the skin. Once in the body, they compete with and displace essential minerals such as Zn, Cu, Mg and Ca, and interfere with organ system function [1]. Heavy metals are especially dangerous because they tend to bioaccumulate, it means that they become toxic when they are not metabolized by the body and accumulate in the soft tissues. The main threats to human health from heavy metals are associated with exposure to lead, cadmium, mercury and arsenic [2].

Stripping techniques are particularly suitable for the determination of trace metals in samples of environmental and biological origin [3], due to their excellent detection limits, their sensitivity to the presence of different metal species, their capacity to multielement determination, and their relatively low cost. The performance of voltammetry is strongly influenced by the working electrode material. For several decades, electrochemical stripping methods were associated with the use of working mercury electrodes due to their wide cathodic potential range [4]. Nowadays, the development of electrochemical biosensors based on chemically modified electrodes for the detection and quantification of metal ions in natural samples with less toxicity and more environmentally friendly than mercury is still a very promising area. An essential aspect in the design of these biosensors is the molecule immobilization procedure [5]. Among others, a strategy for modifying

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4 electrodes is based on the formation of self-assembled monolayers (SAM). Nevertheless, as
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6 reported for peptides or for macrocyclic compounds such as crown ethers, another strategy
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8 that has demonstrated its usefulness for forming stable complexing monolayers is based on
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10 the recognition molecule immobilization on aryl diazonium salt monolayers anchored on
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12 the electrode surface [6-8]. This alternative strategy can overcome the major limitations of
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14 thiol SAMs such as the narrow potential range for metal ion detection [9,10], providing
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16 stable sensors than can be used for a large number of measurements without degradation
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18 signs [7,8].
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23 It is well-known the affinity of some chelators to different cations as Cu^{2+} , Fe^{2+} , Ca^{2+} , Zn^{2+} ,
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25 and Pb^{2+} [11]. Particularly, D-penicillamine (Pen – Cuprimine, Depen), that has long been
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27 used as the drug of choice in the treatment of Wilson’s disease or hepatolenticular
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29 degeneration, is a potential and effective chelator for some transition metals [12-14].
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33 In this paper, we report for the first time the modification and analytical characterization of
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35 a glassy carbon electrode (GCE) with D-penicillamine, which was immobilized on aryl
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37 diazonium salt monolayers anchored to the electrode surface, for the simultaneous
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39 determination of Cd(II) and Pb(II) using voltammetric techniques. Moreover,
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41 penicillamine-GCE sensor was applied for the monitorization of Pb(II) and Cd(II) ions in a
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43 certified wastewater sample.
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53 **2. EXPERIMENTAL**

54 55 56 **2.1 Chemicals**

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4 4-aminobenzoic acid (ABA), *N*-hydroxysulfosuccinimide (sulfo-NHS), *N*-(3-
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6 dimethylaminopropyl)-*N*'-ethylcarbodiimide hydrochloride (EDC), D-penicillamine,
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8 certified reference material, wastewater ERM[®]- CA713, potassium dihydrogen phosphate,
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10 sodium monophosphate, methanol and sodium nitrite were purchased from Sigma-Aldrich
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12 (St. Louis, MO, USA). 2-(*N*-morpholino)-ethanesulfonic acid (MES), potassium
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14 ferrocyanide K₄[Fe(CN)₆]·3H₂O, hydrochloride acid, acetic acid and sodium acetate were
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16 provided by Merck (Darmstadt, Germany). Potassium ferricyanide K₃[Fe(CN)₆] was
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18 supplied from Panreac (Barcelona, Spain). All reagents were of analytical grade. Pb(II) and
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20 Cd(II) stock solutions 10⁻² mol L⁻¹ were prepared from Pb(NO₃)₂·4H₂O and
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22 Cd(NO₃)₂·4H₂O respectively and standardized complexometrically [15]. 0.1 mol L⁻¹ acetic
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24 acid / acetate buffer solution (pH 4.5) was used for pH control. Ultrapure water (Milli-Q
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26 plus 185 system, Millipore) was used in all experiments.
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36 **2.2 Apparatus**

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38 Stripping measurements were performed in a glass cell by means of a μ Autolab System
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40 Type III (EcoChemie, The Netherlands) attached to a Metrohm 663 VA Stand (Metrohm,
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42 Switzerland) and a personal computer with GPES 4.9 software (EcoChemie).
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46 The reference and the auxiliary electrode were Ag|AgCl|KCl (3 mol L⁻¹) and Pt wire,
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48 respectively (Metrohm, Switzerland). The working electrode used was a penicillamine-
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50 GCE prepared from a commercial glassy carbon disk electrode of 2 mm diameter provided
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52 by Metrohm (Switzerland).
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56 A pH meter Basic 20 (Crison Instruments, Barcelona, Spain) was used for pH
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58 measurements.
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4 All measurements were carried out at room temperature (20 °C).
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8 9 **2.3 Procedures**

10 11 **2.3.1 Preparation of glassy carbon disk electrode (GCE)**

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14 Glassy carbon disk electrodes (GCE) were commercially purchased. Prior to their
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16 functionalization, the electrode surface was moistened with MilliQ water and then polished
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18 using a suspension of alumina particles of 300 nm diameter, and then rinsed with water and
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20 sonicated in absolute ethanol and water in order to obtain a reproducible electrochemical
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22 surface.
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26 27 28 29 **2.3.2 Preparation of modified GECs**

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31 The principle of the modification of the GCE is illustrated in Figure 1, with specific steps
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33 described below [16].
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36 37 **2.3.2.1 Diazonium salt electrografting**

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39 The *in situ* generation of the aryl diazonium was performed by adding 2 mmol L⁻¹ of
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41 sodium nitrite to an acidic solution (1 mol L⁻¹ aqueous HCl) of 73 mmol L⁻¹ ABA. These
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43 solutions were mixed for about 30 min in an ice bath, prior to the electrochemical grafting
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45 process [17] conducted by scanning the potential at 0.2 V s⁻¹ from 0 V to -1 V for 100
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47 cycles. The functionalized electrodes were thoroughly rinsed with Milli-Q water and
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49 methanol to remove any physisorbed compounds.
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53 54 55 56 **2.3.2.2 Covalent immobilization of D-penicillamine via carbodiimide coupling** 57 58 59 60 61 62

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4 The carboxyl groups of the electrografted diazonium salt were activated by incubating the
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6 functionalized electrodes in a 26 mmol L⁻¹ EDC and 35 mmol L⁻¹ sulfo-NHS solution in
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8 100 mmol L⁻¹ MES buffer (pH 4.5) for 1 h. The activated groups reacted overnight with the
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10 amine terminal groups of chelator (D-penicillamine at 2.9 mg/100 mL in 0.1 mol L⁻¹ MES
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12 buffer) at 4 °C to form amide bonds.
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19 **2.3.3 Voltammetric measurements**

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21 Before each set of measurements, the electrodes were scanned in acetate buffer solution in
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23 order to get stable voltammetric responses.
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26 Stripping voltammetric determinations using penicillamine-GCE of Cd(II) and Pb(II) were
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28 done at a deposition potential (E_d) of -1.25 V, applied with stirring of 1500 rpm during a
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30 deposition time (t_d) of 120 s and followed for a rest period (t_r) of 5 s. Determinations were
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32 done by scanning potential from -1.25 to -0.5 V using pulse times of 50 ms, step potentials
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34 of 5 mV and pulse amplitudes of 100 mV.
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38 The linear calibration plots for the simultaneous determination on a penicillamine-GCE by
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40 stripping voltammetry of Pb(II) and Cd(II) were obtained by increasing metal
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42 concentrations in pH 4.5 acetic acid / acetate buffer media.
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45 In the analysis of the certified wastewater sample, voltammetric analysis started by placing
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47 in the cell a volume of the sample (ERM[®]- CA713) in 0.1 mol L⁻¹ acetic acid / acetate
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49 buffer solution (pH 4.5) and the voltammetric scan was recorded. In the case of calibration
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51 by standard addition, five aliquots of Pb(II) and Cd(II) standard solution were further added
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53 and the respective curves were recorded. All solutions were mechanically stirred for 1min
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58 at 1500 rpm after each standard addition.
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4 In order to eliminate any remaining bound metals from the electrode, an electrochemical
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6 cleaning stage was considered between measurements. This stage was performed by
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8 applying a conditioning potential (E_{cond}) of -0.5 V for 30 s after each measurement, in a cell
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10 containing fresh buffer solution.
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14 All experiments were carried out without any oxygen removal.
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18 19 **3. RESULTS AND DISCUSSION** 20

21 22 **3.1 GCE characterization** 23

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25 The electrochemical response using 2 mmol L⁻¹ ferrocyanide/ferricyanide as redox probe in
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27 100 mmol L⁻¹ phosphate buffer (pH 7.4) was investigated at each functionalization step
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29 using cyclic voltammetry (CV). CV measurements were performed in unstirred conditions
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31 scanning the potential at 100 mV s⁻¹ from -0.6 to 0.6 V. Electrografting resulted in
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33 decreasing current as expected (Figure 2). Covalent binding of chelator also resulted in
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35 lower current peaks compared to bare electrode, as shown in Figure 2 for penicillamine-
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37 GCE. These observed changes in the voltammograms confirmed the modifications
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39 occurring on the electrode surface.
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46 47 **3.2 Repeatability and reproducibility** 48

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50 In order to ensure the detection of considered metals at penicillamine-GCE sensor in the
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52 selected concentration range; different values of E_d were firstly tested in order to select the
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54 optimum value of the potential for the simultaneous determinations of Cd(II) and Pb(II).
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56 Once the E_d was selected, different stirring rates ranging from 500 to 2500 rpm and t_d
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58 ranging from 30 to 300 s were studied looking for a compromise between the peak currents
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4 and the time of the analysis. The selected conditions were for all cases, an E_d of -1.25 V
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6 applied with stirring of 1500 rpm during a t_d of 120 s and followed for a t_r of 5 s. Two
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8 different pH values 4.5 and 6.0 were also tested for the simultaneous determination of
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10 Cd(II) and Pb(II). At pH value of 6.0 a decrease in the peak current of metal ions was
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12 detected with respect to the obtained at pH 4.5. According to this study, an optimal pH
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14 value of 4.5 was selected.
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18 In order to test the repeatability and reproducibility of the developed penicillamine-GCE
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20 sensor, individual stripping measurements of $112 \mu\text{g L}^{-1}$ of Cd(II) and $60 \mu\text{g L}^{-1}$ of Pb(II)
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22 in pH 4.5 acetic acid / acetate buffer solution by applying the described voltammetric
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24 procedure were carried out. The reproducibility calculated from two different
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26 penicillamine-GCE units modified the same day within a series of ten repetitive
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28 measurements yielded RSD of 3.4 % and 4.1 % for Cd(II) and Pb(II), respectively. In
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30 addition, for Cd(II) the reproducibility calculated from two different penicillamine-GCE
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32 units modified at different days within a series of five repetitive measurements was also
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34 estimated yielding a RSD of 6.2 %. The repeatability estimated using the same
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36 penicillamine-GCE unit for ten repetitive measurements produced RSD of 1.9 % and 2.5 %
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38 for Cd(II) and Pb(II), respectively.
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45 These reproducibility and repeatability values are similar or even better than those reported
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47 for graphite–epoxy composite electrodes (GEC) modified with the same strategy with
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49 crown ethers [8].
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52 Additionally, the durability of such modified electrode was tested from the reproducibility
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54 calculated from two different penicillamine-GCE units, one newly modified and the second
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56 one used after more than forty measurements, within a series of five repetitive
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58 measurements yielding RSD of 3.8 % for Cd(II). This value confirms the use of
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4 penicillamine-GCE for a large number of voltammetric measurements without signs of
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6 deterioration.
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10 11 **3.3 Linearity, limit of detection (LOD) and limit of quantification (LOQ)** 12 13

14 First of all, separate and simultaneous calibration of Cd(II) and Pb(II) ions by stripping
15 voltammetry were carried out on penicillamine-GCE (Figure 3) in order to study if the
16 presence of Cd(II) influences the sensitivity of Pb(II), and vice versa. Sensitivities ($\mu\text{A} / \mu\text{g}$
17 L^{-1}) obtained as the slope value of the individual and simultaneous calibration plots of
18 Cd(II) and Pb(II) were 0.12 and 0.13 for Cd(II), and 0.077 and 0.085 for Pb(II),
19 respectively. At the view of these results, it can be concluded that the presence of Cd(II)
20 does not affect the determination of Pb(II) and vice versa. Therefore, linearity, limit of
21 detection (LOD) and limit of quantification (LOQ) were calculated for the simultaneous
22 determination of Cd(II) and Pb(II) ions on penicillamine-GCE by stripping voltammetry
23 (Table 1). The lowest value of the linear concentration range was established from the
24 corresponding limit of quantification (LOQ). The LOQ was calculated as 10 times the
25 standard deviation of the intercept over the slope of the calibration curve of the target ions.
26 Limit of detection (LOD) was evaluated by considering 3 times the previous relation. For
27 LOD and LOQ determinations, seven standard concentrations of Cd(II) and Pb(II) were
28 used as calibration samples. Figure 3c shows the evolution of DPASV signals of Cd(II) and
29 Pb(II) using the penicillamine-GCE sensor when the concentration of the considered metals
30 simultaneously increases. In all cases, well defined stripping peaks without any evidence of
31 signal splitting were observed over the considered concentration range. Linear calibration
32 curves were obtained for Cd(II) and Pb(II) up to a maximum concentration level of 115 μg
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4 L^{-1} for both considered metal ions. The corresponding regression equations and the
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6 correlation coefficient for both metals are presented in Table 1. As shown in Table 1, both
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8 LOD and LOQ were at the level of $\mu\text{g L}^{-1}$ for Cd(II) and Pb(II) ions, being those obtained
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10 for Pb(II) slightly lower than for Cd(II). It should be mentioned that no previous LOD and
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12 LOQ data for penicillamine-GCE sensor are available in the literature. Nevertheless, in
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14 comparison with previous results achieved using others electrodes modified following the
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16 same strategy, *e.g.*, peptide-modified electrodes [7, 18] or crown ether-modified electrodes
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18 [8], the LOD and LOQ obtained in this work for Cd(II) and Pb(II) are similar or even
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20 slightly lower depending on the modified electrode considered. It should be pointed out that
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22 in this paper the applied deposition time was significantly reduced, being of 120 s instead
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24 of the 300 s used with others modified-electrodes. Therefore, lower concentrations ranges
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26 and better detection limits could still be achieved using the proposed method by increasing
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28 the deposition time. Furthermore, the results achieved using penicillamine-GCE sensor are
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30 also comparable or even better than those reached using some solid composite electrodes,
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32 *e.g.*, graphite solid composite electrode, solid composite silver electrode, carbon composite
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34 solid electrode modified with silica gel... [in Ref. [19], Table 3], or some film electrodes
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36 [20-22].

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38 Therefore the reported calibration data suggests that the penicillamine-GCE sensor could be
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40 fully suitable for the simultaneous determination of Cd(II) and Pb(II) at trace levels in
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42 natural samples. Additionally, penicillamine immobilized on aryl diazonium salt
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44 monolayers anchored on a glassy carbon electrode surface leads to high stable durability
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46 sensors that do not require any previous treatment before starting measurements.
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60 **3.4 Application to the analysis of a wastewater reference material**

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4 A wastewater certified reference material (ERM[®]- CA713) was selected to study the
5 applicability of penicillamine-GCE sensor for the determination of Cd(II) and Pb(II).
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9 Considered metal ions were determined by means of the standard addition method. Then,
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A wastewater certified reference material (ERM[®]- CA713) was selected to study the applicability of penicillamine-GCE sensor for the determination of Cd(II) and Pb(II). Considered metal ions were determined by means of the standard addition method. Then, voltammetric stripping measurements following the above-mentioned conditions were carried out including the additions of Pb(II) and Cd(II). Figure 4a shows representative stripping voltammograms obtained in the analysis of the wastewater samples using penicillamine-GCE. Pb(II) peak behaves in the same way as in the calibration data: a well-defined peak was obtained; whereas for Cd(II) a wider stripping signal was observed.

Standard addition plot for Pb(II) (Figure 4b) shows the good correlation of the representative stripping voltammetric measurements carried out using penicillamine-GCE. It should be mentioned that the Cd(II) standard addition plot is not provided because although the Cd(II) could be perfectly determined simultaneously with Pb(II) using penicillamine-GCE, as it is shown in Figure 3c, the concentration of Cd(II) in the analyzed wastewater sample is below the LOQ.

The Pb(II) concentration data obtained from the voltammetric determination of three replicates of the certified wastewater sample performed using penicillamine-GCE are reported in Table 2. A good concordance between Pb(II) concentrations were obtained, as well as with the certified Pb(II) value. Therefore, these good results confirm the applicability of the developed penicillamine-GCE sensor for the determination of low concentration levels of Pb(II) simultaneously with Cd(II) ions in natural samples, being an interesting and valuable alternative to the most conventional electrodes for the determination of these metal ions.

4. Conclusions

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4 In this work, a new sensor based on the immobilization of penicillamine, as a chelator
5 agent, on aryl diazonium salt monolayers anchored on a glassy carbon electrode surface
6 was successfully developed. This modified electrode constitutes an alternative to the more
7 widespread electrodes for the determination of metal ions such as Cd(II) and Pb(II) in
8 samples of environmental interest. Penicillamine-GCE sensor exhibits a good repeatability
9 and reproducibility and can be used for a large set of measurements without signs of
10 degradation or loss of sensitivity, allowing the simultaneous determination of Cd(II) and
11 Pb(II). Regarding their analytical performance, penicillamine-GCE provides well-shaped
12 stripping peaks for Cd(II) and Pb(II) over the considered concentration range. The achieved
13 LODs and LOQs were at levels of $\mu\text{g L}^{-1}$ for all metals, and similar or slightly lower to
14 those obtained for other electrodes modified following the same strategy, applying
15 deposition time significantly reduced with respect to earlier studies [7, 8, 18].

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17 Under the above optimized conditions, the simultaneous determination of Cd(II) and Pb(II)
18 in a wastewater sample on penicillamine-GCE was successfully attempted being able to
19 quantify the Pb(II) concentration with very high reproducibility and good trueness inferred
20 by the relative standard deviation (1.5 %) and the relative error (2.4 %) respectively.
21 Regarding Cd(II), its quantification was not possible since its concentration in the analyzed
22 wastewater sample was below the obtained LOQ. Nevertheless, these good results confirm
23 the applicability of the developed penicillamine-GCE for the determination of low
24 concentration levels of Pb(II) simultaneously with Cd(II) ions in natural samples.

25 26 27 28 29 30 31 32 33 34 35 36 37 38 39 40 41 42 43 44 45 46 47 48 49 50 51 52 53 54 55 **Acknowledgments**

56
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7 **References**
8

9 [1] E. Lawrence, A.R.W. Jackson, J.M. Jackson, Logman Dictionary of Environmental
10 Science, Addison Wesley Longman, Harlow, UK, 1998.
11

12
13
14 [2] L. Järup, British Medical Bulletin 68 (2003)167–182.
15

16
17 [3] J. Wang, Stripping Analysis: Principles, Instrumentation and Applications, VCH,
18 Deerfield Beach, FL 1985.
19

20
21
22 [4] J. Barek, A. G. Fogg, A. Muck, J. Zima, Crit. Rev. Anal. Chem. 31 (2001) 291–309.
23

24
25 [5] U.E. Wawrzyniak, P. Ciosek, M. Zaborowski, G. Liu, J.J. Gooding, Electroanalysis 25
26 (2013)1461–1471.
27

28
29 [6] J. Gooding, D. Hibbert, W. Yang, Sensors 1 (2001) 75-90.
30

31
32 [7] N. Serrano, B. Prieto-Simón, X. Cetó, M. del Valle, Talanta 125 (2014) 159–166.
33

34
35 [8] N. Serrano, A. González-Calabuig, M. del Valle, Talanta 138 (2015)130-137.
36

37 [9] M. Delamar, R. Hitmi, J. Pinson, J.M. Saveant, J. Am. Chem. Soc. 114 (1992) 5883–
38 5884.
39

40
41 [10] J.J. Gooding, S. Ciampi, Chem. Soc. Rev. 40 (2011) 2704–2718.
42

43
44 [11] C. Gourlaouen, O. Parisel, Int. J. Quantum Chem. 108 (2008) 1888–1897.
45

46
47 [12] J. Peisach, W. E. Blumberg, Mol. Pharmacol. 5 (1969) 200-209
48

49 [13] E. Chekmeneva, J. M. Díaz-Cruz, C. Ariño, M. Esteban, Anal. Chim. Acta 653 (2009)
50 77-85
51

52
53 [14] F. Jalilehvand, B. O. Leung, V. Mah, Inorg. Chem., 48 (2009) 5758–5771
54

55
56 [15] A.I. Vogel, Textbook of Quantitative Chemical Analysis, 5th ed., Pearson Education
57 Limited, Harlow, GB, 1989.
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[16] C. Ocaña, M. del Valle, *Microchim. Acta* 181 (2014) 355–363.

[17] D. Belanger, J. Pinson, *Chem. Soc. Rev.*, 40 (2011) 3995–4048.

[18] D. Ebrahimi, E. Chow, J.J. Gooding, D.B. Hibbert, *Analyst*, 133 (2008) 1090–1096

[19] T. Navratil, J. Barek, *Crit. Rev. Anal. Chem.*, 39 (2009) 131–147

[20] C. Pérez-Ràfols, N. Serrano, J. M. Díaz-Cruz, C. Ariño, M. Esteban, *Electroanalysis* 27 (2015) 1-6

[21] V. Sosa, C. Barceló, N. Serrano, C. Ariño, J. M. Díaz-Cruz, M. Esteban, *Anal. Chim. Acta* 855 (2015) 34-40

[22] N. Serrano, J. M. Díaz-Cruz, C. Ariño, M. Esteban, *Electroanalysis* 22 (2010) 1460-1467

Table 1 Calibration data for the simultaneous determination of Pb(II) and Cd(II) on penicillamine-GCE at E_d of -1.25 V, t_d of 120 s and pH 4.5

	Pb(II)	Cd(II)
Sensitivity ($\mu\text{A} / \mu\text{g L}^{-1}$)	0.085	0.13
R^2	0.999	0.998
Linear range ($\mu\text{g L}^{-1}$) ^a	10.07-115.14	14.34-115.23
LOD ($\mu\text{g L}^{-1}$)	3.02	4.30

^aThe lowest value of the linear range was considered from the LOQ

Table 2 Total concentrations of Pb(II) and Cd(II) determined in certified wastewater (ERM[®] - CA713) by stripping voltammetry on penicillamine-GCE by standard addition calibration method applying an E_d of -1.25 V and t_d of 120 s at pH 4.5

	Lead			Cadmium	
	c ($\mu\text{g L}^{-1}$)	RSD (%)	Relative error (%)	c ($\mu\text{g L}^{-1}$)	RSD (%)
	50.9	1.5	2.4	n.q.	—
Certified metal value	49.7	3.4	—	5.09	3.9

n=3 for RSD (%); n.q. not quantified.

Figure 1

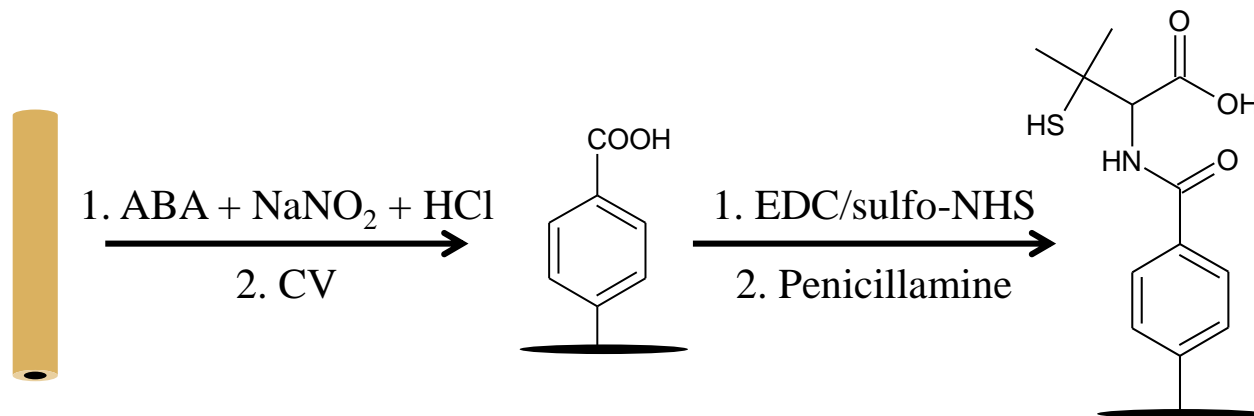


Figure 2

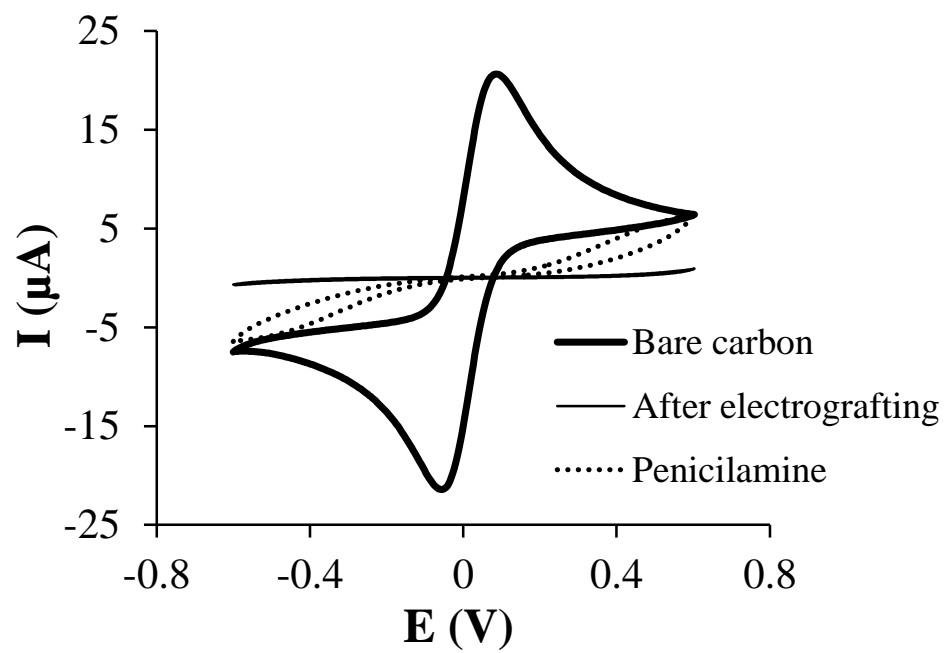


Figure 3

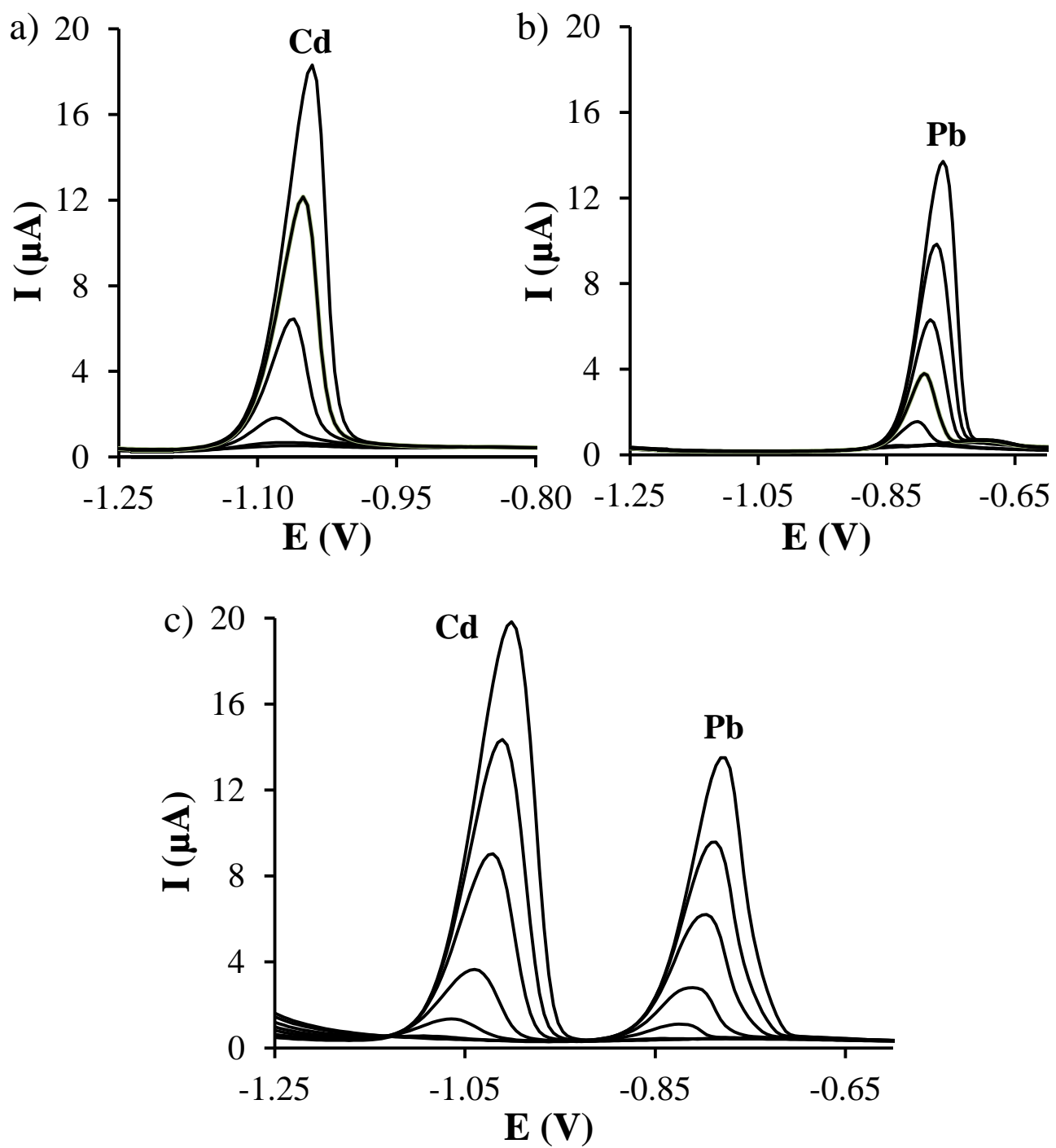
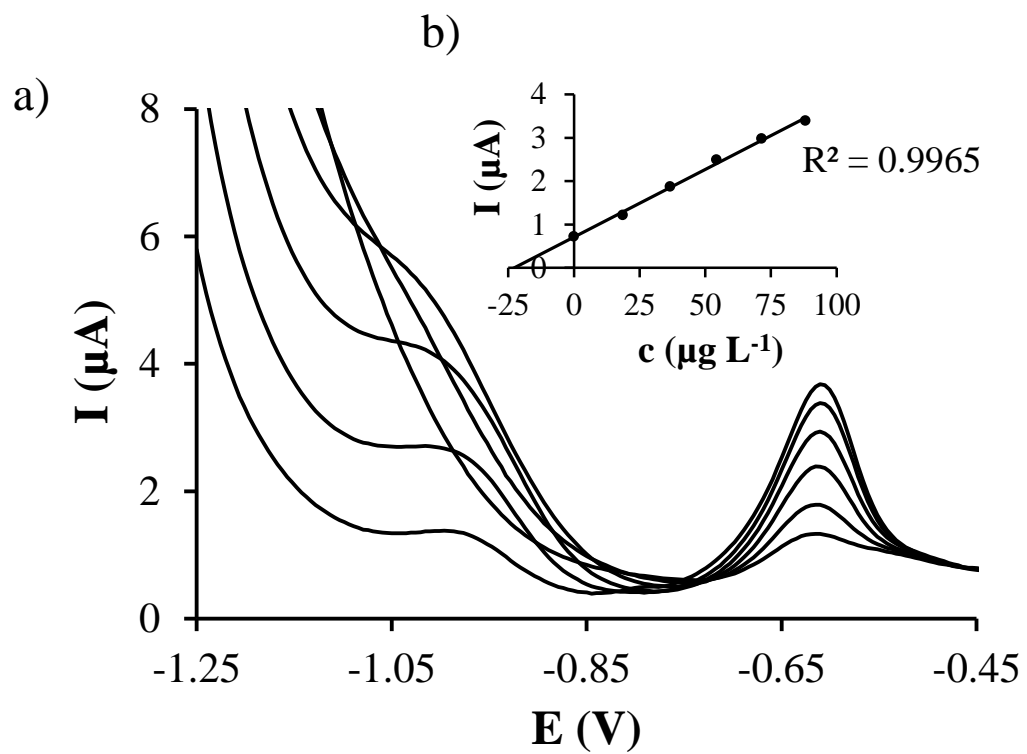


Figure 4



Caption to figures

Figure 1. Scheme of the preparation of penicillamine-GCE by electrochemical grafting.

Figure 2. CVs plots recorded at each electrode functionalization step. Measurements were performed in a 2 mM ferrocyanide/ferricyanide solution in phosphate buffer.

Figure 3. Stripping voltammetric measurements of increasing concentrations ranging from 1.0 to 115 $\mu\text{g L}^{-1}$ of (a) Cd(II), (b) Pb(II), and (c) Cd(II) and Pb(II) recorded on a penicillamine-GCE at pH 4.5 using a E_d of -1.25 V during 120 s and t_r of 5 s.

Figure 4. a) Stripping voltammetric measurements in wastewater samples on penicillamine-GCE at pH 4.5 using a E_d of -1.25 V during a t_d of 120 s and t_r of 5 s; and, b) Pb(II) standard addition plot.