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Can bismuth film screen printed carbon electrodes be used to study complexation?

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ABSTRACT

Ex situ bismuth film on commercial screen-printed carbon electrodes (BiSPCE) has been used for the first time for the analysis of the complexation of Cd^{2+} by thiol containing compounds as glutathione (GSH) and phytochelatin ($(\gamma$-Glu–Cys$)_2$–Gly (PC_2).

Reproducibility of data is quite satisfactory and allows their treatment by multivariate curve resolution by alternating least squares (MCR-ALS). Unitary voltammograms and concentration profiles provided by MCR-ALS confirm the formation of 1:1 and 1:2 Cd^{2+}:GSH and 1:2 Cd^{2+}:PC_2 complexes. These results are in agreement with those previously obtained by mercury electrodes, and allow us to propose the use of BiSPCE for further studies on complexation.

1. Introduction

Glutathione (ɣ-Glu–Cys–Gly, denoted usually as GSH) and its oligomers named phytochelatins ($(\gamma$-Glu–Cys$)_n$–Gly, denoted usually as PC_n, where n generally ranges from 2 to 6) are thiol rich peptides which are synthesized enzymatically by plants in response to an excessive uptake of certain heavy metal ions [1–4]. The great affinity of the thiol group for heavy metals makes important the complexation by these thiol containing peptides not only for heavy metal detoxification but also for phytoremediation purposes [5–7].

The complexation of metals as Cd^{2+}, Zn^{2+}, Pb^{2+} and Cu^{2+} with GSH [8–10], mixtures of GSH and SeCyst [11], and PC_n [8–10,12–19] has already been studied by voltammetric methods in Hg electrodes. The resulting voltammetric data have been analyzed by chemometric methods as multivariate curve resolution by alternating least squares (MCR-ALS) [8–10,14–18] and/or gaussian peak adjustment (GPA) [11,19] and a complexation sequence has been proposed for most of the analyzed systems. However, certain metal-thiol peptides systems present the difficulty that thiol favors the electrodic oxidation of the mercury of the electrode. This process generates anodic signals that are strongly overlapping with those related with the metal ion to be studied, making the analysis of these systems even more complicated [20,21]. By the other hand, a policy against the use of mercury as electrodic material has appeared in the last years due to its potential toxicity.

In the last decade bismuth electrodes have been postulated as a valuable alternative to mercury electrodes for the analysis of traces of heavy metals in environmental samples [22]. Despite the lower toxicity of the bismuth electrodes and their versatility and diversity in use, only a significantly small number of papers is devoted to the heavy metal complexation by voltammetric techniques [23,24]. Particularly, in one of these previous works, the complexation of Pb(II) by GSH has been studied by differential pulse voltammetry (DPV) on an *ex situ* bismuth film electrode (BiFE), achieving a better resolution of the signals than on a mercury drop electrode (SMDE), and allowing a qualitative analysis of the complexation process (not possible on SMDE). In contrast, it was found that because of the more complex shape of the signals obtained for the Pb(II)–GSH system on BiFE, the DPV/MCR-ALS analysis could not be successfully applied and was unable to provide essential information on complex composition [24].

In this work, the study of the complexation of Cd(II)–GSH and Cd(II)–PC_2 on an *ex situ* bismuth film on commercial screen-printed carbon electrodes (BiSPCE) was carried out. Taking into account that BiSPCE increasingly gained advantage over the most conventional BiFE due to the improvement of many aspects, such as the disposable character, the minimised splitting of the metal peaks, their sensitivity and their linearity ranges [25–28], the aim of the present work is the evaluation of the applicability of the BiSPCE in the study of the Cd(II)–GSH and Cd(II)–PC_2 systems, and the subsequent treatment with MCR-ALS or GPA of the obtained data, allowing us the establishment of the stoichiometry of the possible complexes formed.

2. Experimental

2.1. Chemicals

A standard Bi(III) solution 1.000 g L^{-1}, atomic absorption standard solution, was purchased from Fluka. All others reagents used

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were Merck analytical grade. Cd(II) stock solution 10^{-2} mol L^{-1} was prepared from $Cd(NO_3)_2 \cdot 4H_2O$ and standardized complexometrically. Glutathione (GSH), in the reduced form, was provided by Merck. (ɣ-Glu–Cys)$_2$Gly (PC_2), in lyophilized form with a purity $>95\%$, was provided by Genosphere Biotechnologies France. Tris (hydroxymethyl) aminomethane, HCl buffer solution 0.1 mol L^{-1} (pH 7.5 and pH 8.5) and KNO_3 0.01 mol L^{-1} were employed as supporting electrolyte. Ultrapure water (Milli-Q plus 185 system, Millipore) was used in all experiments.

2.2. *Apparatus and measurements*

Differential pulse voltammetric (DPV) measurements were performed on a bismuth screen printed carbon electrode (BiSPCE) in an Autolab System PGSTAT12 (EcoChemie, The Netherlands) attached to a Metrohm 663 VA Stand (Metrohm, Switzerland) and a personal computer with GPES version 4.9 data acquisition software (EcoChemie).

The reference electrode (to which all potentials are referred) and the auxiliary electrode were Ag/AgCl/KCl (3 mol L^{-1}) and Pt wire, respectively.

The working electrode was a BiSPCE prepared from a commercial screen-printed carbon disk electrode of 4 mm diameter (ref. 110, DS SPCE) provided by DropSens (Spain) and connected to the Autolab by means of a flexible cable (ref. CAC, DropSens). A new BiSPCE was used for each voltammetric titration [25].

Ex situ bismuth coated method was selected in order to prevent the side reactions that can take place between Bi^{3+}-ions and ligands.

All the measurements were carried out in a glass cell at room temperature (20 °C) under a purified nitrogen atmosphere (Linde N50) in order to prevent the possible oxidation of the thiol compounds.

Unless otherwise indicated, a pulse of 50 mV and a potential step of 2 mV were applied.

2.3. *Ex situ preparation of BiSPCE*

The SPCE, the reference, and the auxiliary electrodes were connected to the stand and immersed into 20 mL of a 0.2 mol L^{-1} acetate buffer solution (pH 4.5) containing 100 ppm Bi(III). After deaeration of the solution for 10 min, a deposition potential of -0.80 V was applied for 300 s with solution stirring, followed by a rest period (without stirring) of 20 s.

Once the bismuth film was deposited, the three electrodes were rinsed carefully with water and the Bi(III) solution was replaced in the cell by the one to be measured. This methodology was previously tested, and results showed a very high repetitivity and reproducibility [25].

2.4. *Procedure*

Voltammetric titrations of Cd(II) with ligand (direct titrations) started by placing in the cell 20 mL of 1×10^{-5} mol L^{-1} Cd(II) solution in 0.1 mol L^{-1} Tris (hydroxymethyl) aminomethane, HCl buffer solution and 0.01 mol L^{-1} KNO_3. Then, the sample was deaerated with pure nitrogen for 20 min and a DPV curve was recorded. Aliquots containing 4×10^{-4} mol L^{-1} of ligand solution were added and, after purging and mechanically stirring the solution for 1 min, the respective DPV curves were recorded. Voltammetric titrations of 20 mL of 1×10^{-5} mol L^{-1} ligand solution with 4×10^{-4} mol L^{-1} of Cd(II) solution (reverse titrations) were also performed following the same procedure.

2.5. *Data treatment*

The DPV curves were smoothed, base line corrected, and converted into data matrices by means of homemade programs implemented in Matlab [29]. The application of MCR-ALS [30] and GPA [31] methods to the analysis of matrices constituted by voltammetric data have been described elsewhere. MCR-ALS and GPA analyses were carried out through several programs implemented in MATLAB (some of them available at http://www.ub.edu/mcr/.html and http://www.ub.edu/dqaelc/programes_eng.html).

3. Results and discussion

3.1. *Cd(II)–GSH system*

The complexation study of the Cd(II)–GSH system was carried out on BiSPCE at pH values of 7.5 and 8.5. Titrations of 1×10^{-5} mol L^{-1} Cd(II) solution with GSH (direct titration) until a GSH:Cd^{2+} ratio value of 3, and those of 1×10^{-5} mol L^{-1} GSH solution with Cd(II) (reverse titration) until a Cd^{2+}:GSH ratio value of 2.25, were done. In both cases the obtained voltammograms were arranged in a data matrix with as many rows as different [GSH]/[Cd(II)] or [Cd(II)]/[GSH] ratios were considered, and as many columns as the number of registered potentials.

Fig. 1 shows the voltammograms obtained in both direct (Fig. 1a) and reverse (Fig. 1b) titrations at pH 7.5. MCR-ALS and GPA analyses were applied to both voltammetric titrations. At a first inspection several components can be observed in all cases, being every component interpreted as every single electrochemical process which contributes to the measured signal [32]. Thus, component 1 is

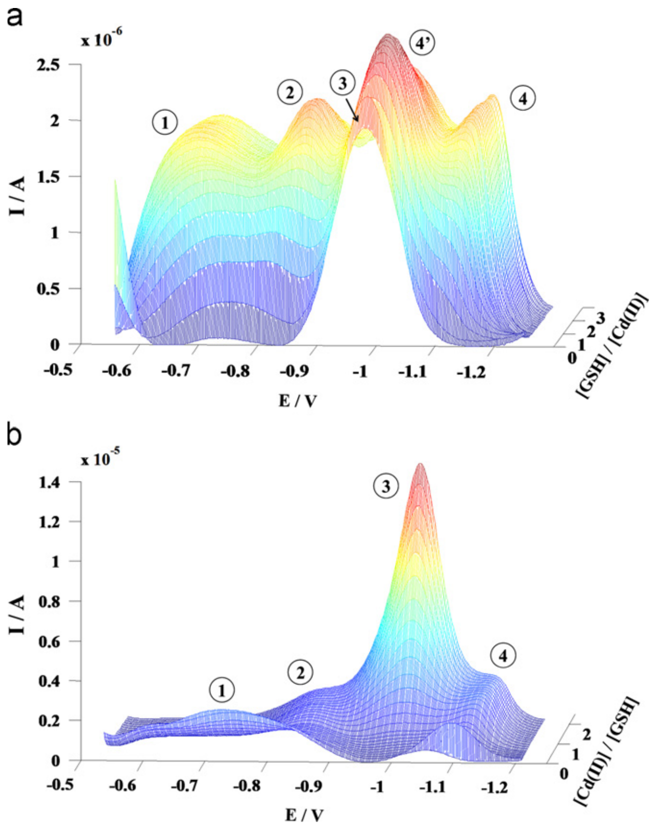

Fig. 1. Experimental data matrix of a 1×10^{-5} mol L^{-1} Cd(II) solution titrated with GSH (a) of a 1×10^{-5} mol L^{-1} GSH solution titrated with Cd(II) (b); both titrations were performed at pH 7.5 and ionic strength 0.01 mol L^{-1} KNO_3.

associated with the anodic signal of the electrode material related with the free ligand [24], component 2 corresponds to an anodic signal, but now related with the formed complexes [20,21,24], component 3 is related with the reduction process of the free metal ion and component 4 and 4′ correspond to the reduction processes of the formed complexes (reduction of the bound metal). Particularly, component 4′, only observed in direct titration, could be associated to a weak Cd(II)–GSH complex, whereas component 4 is related to a more stable Cd(II)–GSH complex.

In the study of Cd(II)–GSH system, direct titration (Fig. 1a) was only used to obtain qualitative information about the processes occurred during the titration, since the strong overlapping between the signal of the free Cd(II) reduction (component 3) and the signal of the weaker Cd(II)–GSH complex (component 4′) does not allow an accurate analysis of the data matrix neither by MCR-ALS nor by GPA. However, the most important information about the complexation processes of the systems is commonly obtained from the data of the reverse titration which, moreover, simulates in a better way a real contamination case of a natural system by Cd(II) ions.

The evolution of the DP voltammograms when Cd(II) is added to a GSH solution (Fig. 1b) shows an initial broad peak (component 1), with a maximum at ca. -0.73 V, attributed to the anodic signal of GSH. With the first additions of Cd(II) this signal decreases and a peak at ca. -1.07 V appears due to the formation of a Cd(II)–GSH complex (component 4). After some additions of Cd(II), the complete disappearance of the anodic signal of the GSH is observed, which allows to observe the presence of a peak at ca. -0.85 V (component 2) that behaves similarly to the signal of the formed complex, indicating its relation with an anodic signal associated with this complex. Finally, at Cd(II)-to-GSH ratios higher than 0.5, it is possible to distinguish a new sharp peak at ca. -1.01 V (component 3) corresponding to the reduction of free Cd(II) at the same time as the signals related with the Cd(II)–GSH complex seem to stabilize.

The general evolution of the Cd(II)–GSH system studied by BiSPCE is equivalent to that obtained in previous works with mercury electrode [33] and, moreover, the currents of the voltammetric signals obtained using the BiSPCE are even higher than those achieved in a mercury electrode because of the largest Bi working surface.

MCR-ALS analysis of the data matrix obtained from the DPV titration of GSH with Cd(II) (Fig. 1b) achieves the best results (lack of fit 9.73%) using 4 components and the constraints of non-negativity (for both concentrations and signals), peak-shape for all components, and selectivity for components 2 and 4. Fig. 2 summarizes the resulting unitary voltammograms (a) and the concentration profiles (b) plotted in front of the Cd(II)-to-GSH ratio.

Although some ambiguities possibly due to the strong overlapping of the signals and the excessive weight of the free metal peak, the analysis of the concentration profile (Fig. 2b) confirms the qualitative behavior of the Cd(II)–GSH system described above. The evolution of the concentration profiles (Fig. 2b) shows that component 1 suffers a progressive decrease from the first addition of Cd(II) that seems to tend to [Cd(II)]/[GSH] ratio of 0.5, although it does not disappear completely. The persistence of component 1 at higher Cd(II)-to-GSH ratios could be due to the fact that its asymmetric peak shape (see unitary voltammograms of component 1 in Fig. 2a) together with the overlapping of signals of components 2–4 causes some ambiguities in the MCR-ALS treatment. On the contrary, the concentration profile of component 3 attributed to free Cd(II) starts to appear at ratio ca. 0.5, supporting the formation of the $Cd(GSH)_2$ complex. But, if only the 1:2 Cd^{2+}:GSH complex was formed, the concentration profile of component 4 (Cd(II)–GSH complexes) should be stabilized at [Cd(II)]/[GSH] ratio around 0.5. The stabilization of this component at ratios ca. 1 indicates that the 1:1 Cd^{2+}:GSH complex could be also formed when an excess of cadmium is present in the medium. Similar conclusions were obtained from the analysis of the titrations of the Cd(II)–GSH system at pH 8.5 (data not shown). This behavior is in accordance with the existing results obtained using a mercury electrode where a model for the complexation of Cd(II) by GSH involving the formation of $Cd(GSH)_2$ and $Cd_2(GSH)_2$ was proposed [33]. This good agreement between BiSPCE and mercury electrode results points out the possibility to use this environmentally safe device to follow easily the complexation process of Cd(II)–GSH system.

3.2. Cd(II)–PC_2 system

A similar study was accomplished to investigate the complexation of Cd(II)–PC_2 system on BiSPCE. Both direct (addition of the peptide to a 1×10^{-5} mol L^{-1} Cd(II) solution) and reverse (addition of Cd(II) to a 1×10^{-5} mol L^{-1} peptide solution) DPV titrations were carried out at pH 8.5. As in the case of Cd(II)–GSH system, direct titration (data not shown) provides qualitative information about the behavior of the system. The evolution of the DP voltammograms obtained in the reverse titration of the Cd(II)–PC_2 system (Fig. 3a) shows an initial wide peak (component 1) at ca. -0.98 V associated with the anodic signal of the electrode material related with the free PC_2. After successive additions of Cd(II), this peak is decreasing and simultaneously a new peak (component 3), at ca. -1.30 V, is increasing. This peak is related with the Cd(II)–PC_2 complex. Finally, a peak at

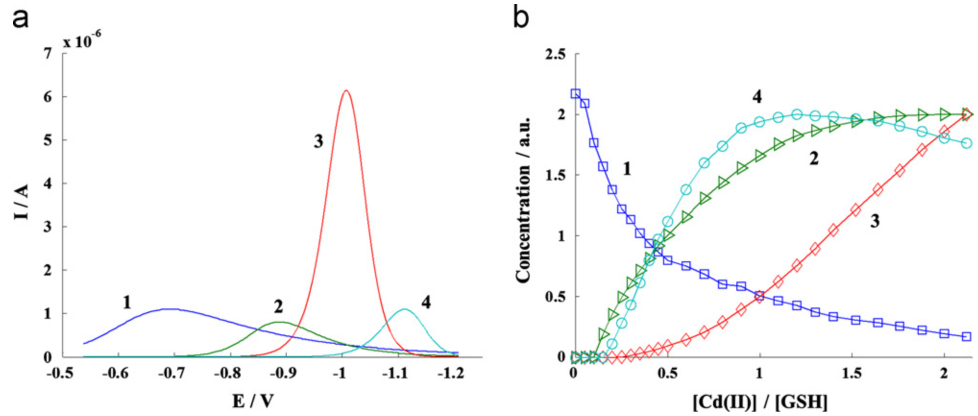

Fig. 2. Normalized unitary response vectors (a) and concentration profiles (b) obtained in the MCR-ALS decomposition of DPV data matrix shown in Fig. 1(b). Components assignments: (1)—anodic signal of the free GSH; (2)—anodic signal of the Cd(II)–GSH complexes; (3)—Cd(II)-ion; (4)—Cd(II)–GSH complexes.

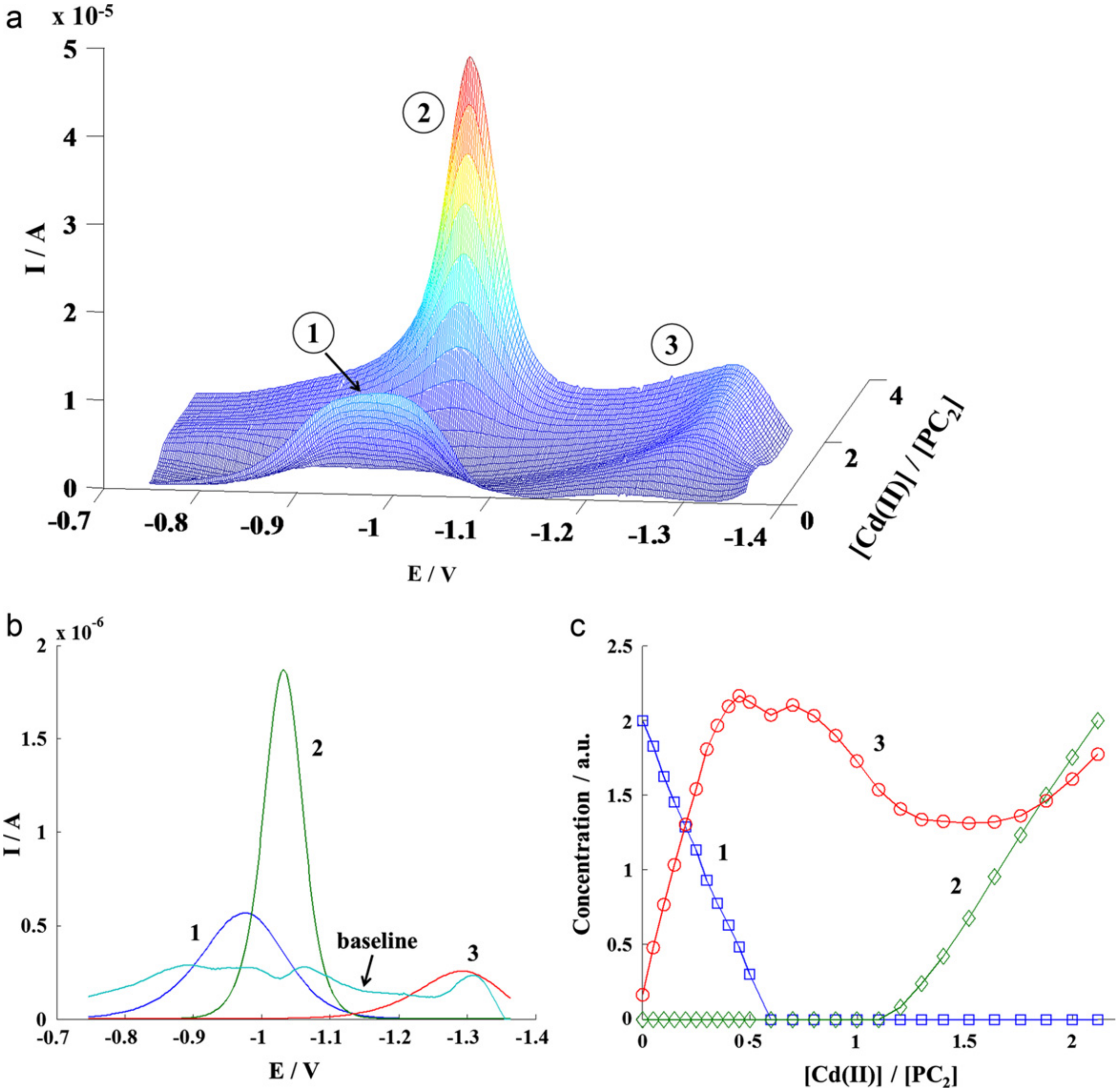

Fig. 3. (a) DPV voltammograms obtained in the titration of 1×10^{-5} mol L^{-1} PC$_2$ solution with Cd(II) at pH 8.5 and ionic strength 0.01 mol L^{-1} KNO$_3$. (b) normalized unitary response vectors estimated from the result of a previous unrestricted MCR-ALS analysis and optimized along shape-restricted MCR-ALS procedure. (c) concentration profiles obtained by MCR-ALS. Components assignments: (1)—anodic signal of the free PC$_2$; (2)—Cd(II)-ion; (3)—Cd(II)–PC$_2$ complex.

ca. -1.03 V, which is attributed to the free metal ion (component 2), is growing with the addition of Cd(II). If we compare the behavior of this system with the previous Cd(II)–GSH one, some differences are appreciated: (i) the anodic signal of the electrode material related with the free PC$_2$ (component 1) and the signal associated with the Cd(II)–PC$_2$ complex (component 3) appear at more negative potential values than those equivalent in the Cd(II)–GSH system. This is due to the higher stability of the binding between Cd(II) and PC$_2$ in comparison with Cd(II)–GSH since, in general, the more negative the reduction peak potential value, the stronger the metal–ligand bond [10]; and ii) the peak related with the anodic signal of the complexes (component 2 in the Cd(II)–GSH system, Figs. 1 and 2) appears only as a distortion of the baseline due to the more irregular shape of the voltammograms obtained in the case of the Cd(II)–PC$_2$ system.

The application of the MCR-ALS analysis to the data matrix obtained in the titration of PC$_2$ with Cd(II) solution revealed that neither considering three components (the anodic signal of the ligand, the signal of the free metal ion and the signal of the Cd(II)–PC$_2$ complex) or four components (the anodic signal of the ligand, the signal of the free metal ion, the signal of the Cd(II)–PC$_2$ complex and its anodic signal) convergence was reached. Nevertheless, a lack of fit of 10.92% was achieved if apart from the three basic components an additional component related with the baseline was considered. The addition of this component, that has not a peak shape, is useful for the correction of the distortion of the original baseline and can be justified after a careful observation and a preliminary analysis of the data matrix (Fig. 3a). The constraints of non-negativity for concentrations and voltammograms, peak shape for all components except the component associated with the baseline, and selectivity for components 1 and 2 were applied in this data treatment. The unitary voltammograms and the concentration profiles provided by MCR-ALS are shown in Fig. 3b and c, respectively. Component 1 disappears at the same time that component 3 increases, until a Cd(II)-to-PC$_2$ ratio of 0.5, in which component 3 progressively stabilizes and component 1 completely disappears suggesting, in agreement with previous studies [10,18], the formation of 1:2 Cd^{2+}:PC$_2$ complex. However, component 2 does not appear until a Cd(II)-to-PC$_2$ ratio about 1, suggesting the formation of a 1:1 or 2:2 Cd^{2+}:PC$_2$ complex whose signal is not visible. This unusual fact could be explained taking into consideration the intricate shape of the DP voltammograms that required the introduction of a baseline component in the MCR-ALS treatment that could hide a too small or too wide signal of such 1:1 complex.

Finally, these results confirm again the goodness of the BiSPCE for the following of the complexation process of the Cd(II)–PC_2 system.

4. Conclusions

Ex situ BiSCPE appears to be a valuable alternative to both mercury and BiFE electrodes for the electrochemical and chemometric study of metal complexation by thiol-rich peptides, as suggested by the study of Cd(II)–GSH and Cd(II)–PC_2 system. On the one hand, bismuth based electrodes present the advantage of being environmentally friendly with quite close features to mercury ones. On the other hand, BiSPCE, as compared to BiFE, does not present any signal splitting for the Cd(II) ion and, because of the accessible and low-cost character of screen-printed electrodes, ensures a user-friendly methodology.

Moreover, the described voltammetric behavior of the Cd(II)–GSH and Cd(II)–PC_2 systems on BiSPCE is similar to that observed on mercury electrodes. Furthermore, MCR-ALS analysis has been applied for the first time to a data matrix obtained on BiSPCE providing abundant information about the complexation process of the systems. Despite some uncertainties attributed to the intricate shape of the obtained voltammograms, especially in the case of the Cd(II)–PC_2 system, the formation of the 1:2 and 1:1 Cd^{2+}:GSH and 1:2 Cd^{2+}:PC_2 complexes was observed, in full agreement with the results obtained with mercury electrodes.

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