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SCREEN PRINTED ELECTRODES USED FOR DETECTION OF IONIC HEAVY METALS

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Abstract: *Electrochemical sensors and biosensors based on inhibition are sensitive methods to determine the concentration of toxic substances in environment and clinic applications. Heavy metal cations bind to biological molecules and inactive important enzyme systems, such as acetylcholinesterase (AChE). In this paper, the amperometric sensors and biosensors based on AChE inhibition were developed using TCNQ modified screen-printed electrodes. The characteristics of the biosensors for different heavy metal ions (copper, cadmium, nichel) detection from aqueous solutions were optimized and evaluated by cyclic voltammetry and amperometry.*

Key words: *electrochemistry, screen printed electrodes, nickel, copper, cadmium.*

1. Introduction

Heavy metal toxicity can result in damaged or decreased mental and central nervous function [9], and damage of blood composition [4], lungs [10], kidneys [23], [18], livers [19], and other important organs [12], [14].

Long-term exposure may result in slowly progressing physical, muscular, and neurological degenerative processes similar to Alzheimer's disease [10], Parkinson's disease [5], muscular dystrophy and multiple sclerosis [24].

The enzymatic activity of acetylcholinesterase (AChE) has been shown to be altered by environmental contaminants such as metals. However, the available literature illustrates a background of contradictory results regarding these effects. Several studies investigated the potential of five metal ions (nickel, copper, zinc, cadmium and mercury) to inhibit AChE activity in vitro, using Ellman's assay, has been studied by Frasco and collaborators [7]. They observed that several metals react with the products of this photometric technique, inducing some artefactual contributions of the interaction of the metals with the technique when measuring AChE inhibition. Under these conditions, the results indicate that with the exception of nickel, all tested metals significantly inhibit AChE activity.

Sensitive and stable monitoring of heavy metals in different matrices (ground water, seawater, food matrices, soils) using screen-printed electrodes (SPE) have been developed.

The application of screen printed electrodes (SPE) on electroanalytical chemistry increased the number of

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procedures for obtaining sensors and enzyme based biosensors (using different enzymes immobilized on SPE). These nanomaterials-based analytical techniques are used for the detection of major families of environmental pollutants, i.e., organic contaminants, heavy metals and air pollutants [11].

A dsDNA-electrochemical biosensor, employing differential pulse voltammetry, was used for the in situ evaluation of Pb^{2+}, Cd^{2+} and Ni^{2+} interaction with dsDNA. The results confirm that Pb^{2+}, Cd^{2+} and Ni^{2+} bind to dsDNA, and that this interaction leads to different modifications in the dsDNA structure. The Pb^{2+} bound to dsDNA can still undergo oxidation. The interaction of Cd^{2+} and Ni^{2+} causes conformational changes, destabilizing the double helix, which can enable the action of other oxidative agents on DNA [17].

The analytical performance of SPE coupled with square wave anodic stripping voltammetry (SWASV) for the simultaneous determination of Pb and Cd in seawater samples, in the low microgL (-1) range, has been evaluated by Guell and collaborators [8].

A new fluorescence sensor for the highly selective detection of Cu^{2+} ion with a detection limit of 3.6 nM based on the aggregation-induced fluorescence quenching of the highly fluorescent glutathione-capped gold nanoparticles is reported [3]

A bi-electrode array (ISE array) has been used in the simultaneous analysis of free copper (II) and iron (III) in seawater without fear of cross-interference between the solid-state sensors [6].

The voltammetric assay of Cu (II) was investigated using a carbon nanotube electrode (CNE) and fluorine immobilized onto a carbon nanotube electrode (FCNE) in cyclic voltammetry (CV), square-wave (SW) stripping voltammetry, and chronoamperometry [15]. The sensor was applied to tap water, blood, and rat tail vascular (in vivo). It was found that the sensor could be used with an interface system in the assay of live cells and non-treated blood.

Using a Cu (2+)-dependent DNA ligation DNAzyme, a colorimetric sensor for Cu^{2+} has been developed based on directed assembly of DNA-functionalized gold nanoparticles by the ligation product, and such ligation DNAzyme-based sensors are intrinsically more sensitive than cleavage DNAzyme systems due to the lack of background [13].

2. Materials and Methods

2.1. Reagents, Samples and other Materials

Acetylcholinesterase enzyme from electric eel (ee), specific activity of 8000 AU.mg-1, acetylthiocholine chloride (ATChCl) substrate and 5,5'dithio (2-nitrobenzoic acid) (DTNB) were purchased from Sigma Chemicals Co (St Louis, MO). AChE stock and working solutions were prepared by dissolution of the enzyme in phosphate buffer saline and solution and kept in a freezer for a maximum of two months. Substrate solutions were prepared by dilution with phosphate buffer solution (PBS), pH 7.5.

Printing pastes (Electrodag PF-410, 423SS, and 6037SS) were purchased from Acheson (Plymouth, UK) and the mediator TCNQ from Aldrich Co (Steinheim, Germany). Graphite T15 was supplied by Lonza A. G. (Basel, Switzerland). The surface area of both reference and working electrode was 0.17 cm^2. The screen-printed electrodes containing TCNQ as mediator used in this study were prepared according to procedures previously described [1], [16].

2.2. Apparatus and Instruments

Screen-printed electrodes (SPEs) were prepared in University of Perpignan Via

Domitia (France) in a three-electrode configuration, 24 electrodes per sheet [2]. A DEK 248 screen-printing system (DEK, Grande-Bretagne) was used for fabrication of the electrodes. The working electrode is a circle with 4 mm diameter which contains TCNQ as electrochemical mediator, the auxiliary electrode is a 16x1,5 mm curve line surrounding on two-sides the working electrode and the Ag/AgCl pseudo-reference electrode is a 5x1.5 mm straight line positioned on the third side of the working electrode [22].

Spectrophotometric measurements for enzyme activity analysis were carried out using a PG Instruments spectrophotometer. Cyclic voltammetry studies were carried out using an electrochemical analyser Autolab PGSTAT12 (Eco-Chemie, The Netherlands).

3. Results and Discussion

In this paper the TCNQ modified screen-printed electrodes are used as sensors for the heavy metal ions detection (Ni^{2+}, Cu^{2+} and Cd^{2+}) and two electrochemical techniques have been used for this purpose, such as cyclic voltammetry and amperometry.

Using cyclic voltammetry the current intensity through the circuit is recorded when the potential is scanned between two appropriate values. The current is due to the redox reactions that are occurring at working electrode surface and which are depending on potential values. The Fig. 1 shows the cyclic voltammetries for TCNQ modified SPEs in different concentrations of Ni^{2+} in NaCl 0.9% the potential is scanned between -0.5 V and 0.7 V at a scan rate of 50 mV s^{-1}.The voltammogram shapes are characteristic to TCNQ which present the peaks that correspond to the oxidation and reduction TCNQ forms. It can be observed that the intensity of the peaks is decreasing function of Ni^{2+} concentrations, showing that TCNQ mediate the redox reaction of the ions.

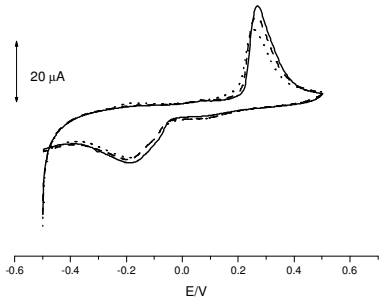

Fig. 1. *Cyclic voltammetry of TCNQ modified SPEs in: (—) 40 µM Ni^{2}+ solution, (----) 60 µM Ni^{2+} solution, (····) 80 µM Ni^{2+} solution. Scan rate 50 mV·s^{-1}*

Other electrochemical technique used to detect the heavy metal ions is the amperometry. In this method the working electrode is kept at a fix value, specific to the analyte, but also depending on electrode material and presence or absence of the electron mediator (such as TCNQ). In the Figure 2 can be seen that Cu^{2+} are detected amperometric. A potential was applied to the electrode and the baseline was allowed to stabilize. The influence of applied potential on Cu detection at TCNQ modified screen-printed electrodes was studied and an optimal potential of 0.1 V vs. Ag/AgCl was found (results not shown). This is in agreement with previous works with TCNQ electrodes and it is ensures a minimizing of interference effects when the electrode is used in real and complex matrices. Aliquots of Cu^{2+} stock solution were added, with continuous stirring, to the cell such that each addition resulted and 1.3 mM increment concentration; the corresponding decrease in current was recorded that correspond to the oxidation of Cu, from which the

baseline was subtracted. The corresponding regression equation of the linear plot was I/µA=0.53c+0.03, R=0.999, where c is the Cu^{2+} concentration in mM.

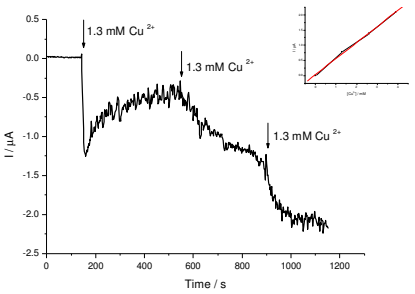

Fig. 2. *Amperometric detection of Cu^{2+} for TCNQ modified SPEs at applied potential 0.1V versus Ag/AgCl. In the insert is shown the typical calibration*

The heavy metal ions are also detected also amperometric in the presence of an enzyme that increase the selectivity of the sensor. Acetylthiocholine chloride (ATChCl) is the substrate of acetylcholinesterase enzyme (AChE) and the enzymatic products are electrochemically detected at the electrode surface. Several studies investigated the potential of metal ions to inhibit AChE activity in vitro. For this purpose this can be used as selective indicator for metal ions such as Ni^{2+}, Cu^{2+} and Cd^{2+} recording the decreasing of electrochemical signal resulted from enzymatic reaction obtained after the enzyme was incubated with heavy metals.

Measurements were performed at fix potential, after stabilization of the baseline, by adding of ATChCl into the solution containing the enzyme and TCNQ modified screen-printed electrodes with continuous stirring. The applied potential on ions detection at TCNQ modified screen-printed electrodes was 0.1 V versus. Ag/AgCl. This is in agreement with previous works with TCNQ electrodes and it ensures a minimum of interference effects when the electrode is used in real and complex matrices.

The effect of incubation time of Ni^{2+}, Cu^{2+} and Cd^{2+}on free AChE (in solution) at TCNQ modified screen-printed electrodes was studied and an optimal time of 10 min was found (results not shown), followed by the addition of the substrate.

Aliquot of 50 µl ATChCl stock solution was added to the cell; the corresponding decrease in current was recorded, from which the baseline was subtracted.

The type of metal ions is important and from Figure 3 can be observed that Cu ions significantly inhibit the AChE activity. There is a big decreasing of the current obtained at the addition of the substrate toward the reference compared with that observed when Ni and Cd ions are used, at the same incubation time and ions concentration. Also, for Cu^{2+} incubation the current is decreasing proportionally with the concentration of inhibition ion concentration as can be seen in Figure 4.

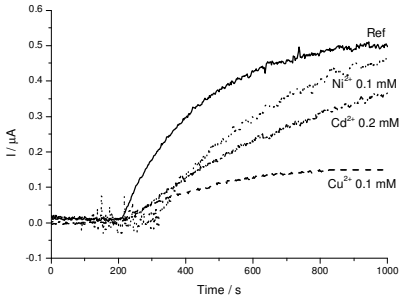

Fig. 3. *Amperometric response for ATCh reaction with AChE inhibited with (----) Cu^{2+}, (····) Ni^{2+} and (-·-·-) Cd^{2+} at TCNQ modified SPEs. Applied potential 0.1V versus Ag/AgCl .*

The corresponding regression equation of the linear plot was I/µA=18.02c+0.07, where c is the Cu^{2+} concentration in mM.

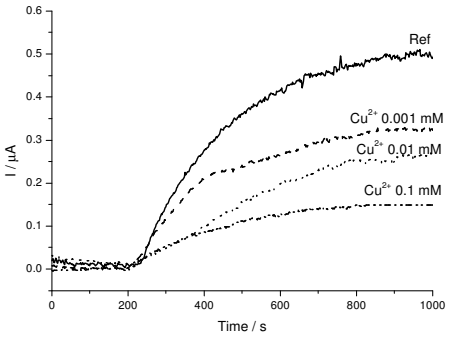

Fig 4. *Amperometric response for ATCh reaction with AChE inhibited with different concentrations of Cu^{2+} at TCNQ modified SPEs. Applied potential 0.1V versus Ag/AgCl*

4. Conclusion

In this paper it has been studied the detection of heavy metal ions at TCNQ modified screen-printed electrodes without and in the presence of AChE enzyme. The enzymatic detection of the heavy metal ions at TCNQ modified screen-printed electrodes has been more sensitive. The sensibility of the sensor was 18.02 µA/mM in the presence of AChE compared with 0.53 µA/mM obtained for the TCNQ modified screen-printed electrode when the ions where detected directly. This results show that the TCNQ modified screen-printed electrodes can be used together with AChE enzyme to detect selectively and with high sensitivity the presence of heavy metal ions. This study represents a start for a future research work in order to obtain an AChE-based biosensor for heavy metal ions detection in real sample, where the enzyme will be immobilized on the electrode surface.

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