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A. Soussou, I. Gammoudi, F. Moroté, M. Mathélié-Guinlet, A. Kalboussi, et al.. Amperometric Polyphenol Biosensor Based on Tyrosinase Immobilization on CoAl Layered Double Hydroxide Thins Films. *Procedia Engineering*, 2016, 168, pp.1131-1134. 10.1016/j.proeng.2016.11.371 . hal-01477810

HAL Id: hal-01477810

<https://hal.science/hal-01477810v1>

Submitted on 3 Mar 2017

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30th Eurosensors Conference, EUROSENSORS 2016

Amperometric Polyphenol Biosensor Based on Tyrosinase Immobilization on CoAl Layered Double Hydroxide Thins Films

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Abstract

An amperometric biosensor based on tyrosinase immobilized on the sensor surface has been used for the detection of polyphenols extracted from green tea. The immobilization was ensured by the crosslinking method on thins films of CoAlSO₄ layered double hydroxide recovering screen-printed gold electrodes. Electrochemical measurements show that this biosensor is able to detect tea polyphenols by following the reduction of compounds enzymatically generated. Its response is linear in the concentration range of [0 - 2,4 μM] with high sensitivity and stability, since it retains 90% of its original response after 20 days.

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Peer-review under responsibility of the organizing committee of the 30th Eurosensors Conference

Keywords: amperometric biosensor; layered double hydroxide; tyrosinase; atomic force microscopy

1. Introduction

Polyphenols are in abundance in our diet, being present in various fruits or vegetables, tea, wine... They play a probable role in the prevention of various diseases [1]. Consequently, intensive studies are carried out to develop innovative methods of detection such as enzyme-based biosensors. Among these enzymes, tyrosinase extracted from mushroom (polyphenol oxidase EC 1.14.18.1) is widely used, this enzyme catalyzing the oxidation of monophenols and o-diphenols to quinones in the presence of oxygen. Amperometric biosensors based on tyrosinase immobilized on various supports recovering the transducer surface have been already reported [2]. Among all strategies available, inorganic matrixes made of layered double hydroxides or HDLs (materials consisting of cationic layers separated by water layers containing anions) appear to be efficient and biocompatible substrates [3]. In our case tyrosinase was immobilized by glutaraldehyde crosslinking method on thins films of CoAlSO₄-LDH recovering screen-printed gold

electrodes (AuSPE). HDL was first synthesized by the coprecipitation method and characterized by Fourier Transform InfraRed (FTIR) and Raman spectroscopies, and X-ray diffraction (XRD). Then the morphological characteristics of LDH thin films (topography, roughness, thickness) were optimized for enzyme immobilization, using atomic force microscopy (AFM), leading to the experimental conditions used in this work. Finally, polyphenol detection was controlled by electrochemical measurements (cyclic voltammograms and chronoamperometry), leading to the conclusion that our biosensor is able to detect polyphenols by the reduction of compounds enzymatically generated, with a high sensitivity probably related to the presence of Co electroactive ions.

2. Experimental section

Salts for CoAlSO₄-LDH synthesis, glutaraldehyde, tyrosinase from *Agaricus bisporus* mushroom (activity ≥ 1000 units/mg) and phosphate buffer saline PBS (10 mM, 0.137 M NaCl, pH 7.4) containing potassium (2.7 mM) were purchased from Sigma-Aldrich. Polyphenols from green tea (product number 193756) were provided by MP Biomedicals LLC (France). Muscovite mica substrates for AFM experiments were purchased from Electron Microscopy Sciences (USA). AuSPEs for electrochemical studies were purchased from Metrohm (ref. 061208210, France).

CoAlSO₄-LDH layers were deposited by spin coating on mica (more suitable for AFM experiments) or AuSPEs, using a solution (15–20 μL) at a concentration of 0.75 mg/mL. Unbounded LDH particles were removed by rinsing with ultrapure water, before tyrosinase deposition (10 μL in buffer, 1.5 mg.mL⁻¹). After incubation at 37°C during 10 min, substrates were overnight-stored in saturated PBS atmosphere at 4°C. Finally, enzyme molecules were reticulated in saturated glutaraldehyde atmosphere for 8 minutes and substrates were rinsed carefully with PBS.

XRD measurements were performed on a Panalytical X'Pert Pro diffractometer using CuK α radiation ($\lambda = 1.5406$ Å) at 40 kV, 30 mA. FTIR spectra of LDHs (in powder) were recorded using a Perkin Elmer spectrophotometer in reflexion mode in the 4000–400 cm⁻¹ range. AFM study was performed by a MultiMode NanoScope II apparatus (AFM imaging) on ambient conditions (air) using a cantilever (with a nominal spring constant of about 40 N.m⁻¹) and at a scan rate between 0.1 and 0.5 Hz. Electrochemical measurements were carried out by using the “910 PSTAT mini” from Metrohm (compact portable potentiostat) at room temperature.

3. Results

3.1. Structural and spectroscopic characterization of CoAlSO₄-LDH

The powder XRD pattern of CoAlSO₄ LDH, shown in Fig. 1a, displays the characteristic diffraction peaks (003, 006, 012, 110 and 113) of pure CoAl-LDH compounds, and is very similar to the XRD pattern already reported [4]. The structure is rhombohedral with refined lattice parameters $a=b=0.3067$ nm and $c=2.2646$ nm, which are consistent with those previously published in the case of CoAl-LDH materials [4].

FTIR spectrum of CoAlSO₄-LDH shown in Fig. 1b reveals the presence of some significant bands. Vibrations at 550 cm⁻¹, 569 cm⁻¹ and 773 cm⁻¹ can be assigned to metal-oxide (Co and Al-O bonds) and metal-hydroxyl vibration modes, respectively. Bands observed at 1350 cm⁻¹ and 1598 cm⁻¹ are assigned to the symmetric stretching of carbonate anions, coming from CO₂ molecules physisorbed onto the surface [5, 6]. At last, at higher wavenumber, the broad band centered at 3373 cm⁻¹ corresponds to the stretching modes of hydroxyl groups, revealing the presence of hydrogen-bonded interlayer water, usually observed around 3300 cm⁻¹ [5]. Raman spectrum confirms in particular the presence of sulfate groups by a band at 471 cm⁻¹ (data not shown).

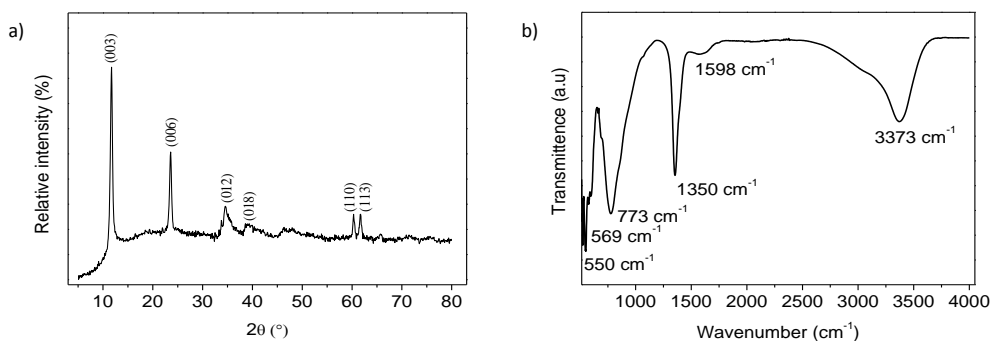


Fig. 1. XRD pattern (a) and FTIR spectrum (b) of $\text{CoAlSO}_4\text{-LDH}$. The main Miller indices are indicated in (a).

3.2. Morphological characterization

Fig. 2a presents the AFM height image of a $\text{CoAlSO}_4\text{-LDH}$ film deposited on mica. The surface is homogeneous containing some localized aggregates, the corresponding average surface roughness being around 5 nm. Scratching operations performed with AFM tip in contact mode on different places of this sample show a film thickness of 10 nm. After tyrosinase immobilization (Fig. 2b), clear changes are observed on the film surface. The roughness and the thickness increase, being estimated around 20 nm and 25 nm, respectively. These data confirm the presence of tyrosinase on $\text{CoAlSO}_4\text{-LDH}$ films, validating the functionalization procedure, which was followed identically to functionalize Au-SPE surfaces.

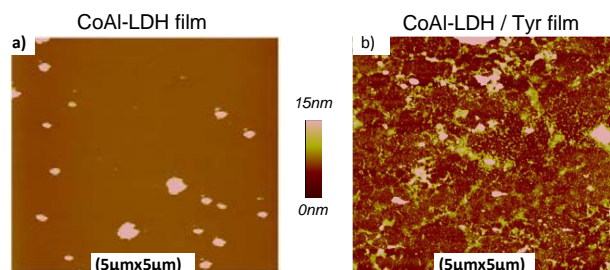


Fig. 2. AFM height images in tapping mode of: (a) $\text{CoAlSO}_4\text{-LDH}$ film ; (b) $\text{CoAlSO}_4/\text{tyrosinase}$ film.

3.3. Electrochemical characterization

The response of the biosensor was tested by adding successive amounts of polyphenols in PBS, concentrations ranging from 0 to 2000 ng/mL. Cyclic voltammograms, recorded in the $[-0.90 \text{ V}; +0.50 \text{ V}]$ range show in particular the presence of a reduction peak at -0.25 V (Fig. 3a showing a “zoom” centered on this peak), assigned to the reduction of o-quinones enzymatically generated from polyphenols [7]. The increase of polyphenol concentration increases the current intensity measured at this potential value. This last point is confirmed by chronoamperometry measurements shown in Fig. 3b. The intensity increase is linear with polyphenol concentration within the range 0.01-1000.00 ng/mL, as shown by corresponding calibration curves at -0.25 V (inset curves in Fig. 3a and 3b). Taking into account the average molar mass of polyphenols of green tea around 415 g/mol determined from tea analysis [8], it gives a linear range of 0-2.4 μM . The slopes are calculated to $2.74 \mu\text{A}/\text{ng}\cdot\text{mL}^{-1}$ ($1137 \text{ A}\cdot\text{M}^{-1}$) and $1.60 \mu\text{A}/\text{ng}\cdot\text{mL}^{-1}$ ($664 \text{ A}\cdot\text{M}^{-1}$) for cyclic voltammetry and chronoamperometry measurements, respectively, with detection limits around 0.30 $\mu\text{g}/\text{mL}$ and 0.10 $\mu\text{g}/\text{mL}$. Such characteristics place our biosensor in the most efficient ones in terms of sensitivity and detection limits. At last, the biosensor is characterized by a good stability, retaining 90% and 58% of its original response after 20 days and one month, respectively. The efficiency of the biosensor

could be due to the presence of Co ions, known for their electroactive properties and their activating action on tyrosinase [3, 9].

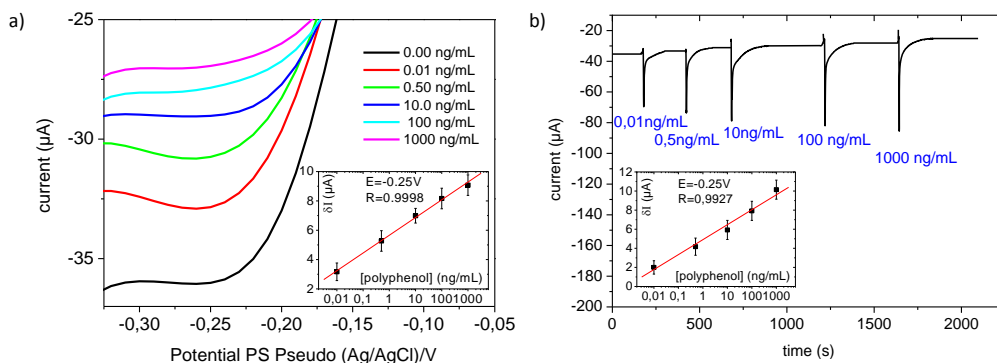


Fig. 3. (a) Cyclic voltammograms and corresponding calibration curve. (b) Chronoamperometry measurements and corresponding calibration curve. ΔI is the difference between the current measured after each polyphenol injection and the initial current without polyphenol.

4. Conclusion

In this work, we described the elaboration of a polyphenol biosensor based on the immobilization of tyrosinase on $\text{CoAlSO}_4\text{-LDH}$ matrix. The resulting biosensor shows remarkable properties such as high sensitivity within a dynamic concentration range of 0-2.4 μM , and good storage stability.

Acknowledgements

Authors thank Gérard Couturier (LOMA) and the NSI platform of LOMA (CPER COLA2) for using the AFM equipment and for technical help, and the Tunisian Ministry of Higher Education and Scientific Research funding for its financial support. They also thank Denise Mondieig and Philippe Négrier (LOMA) for helpful discussion on XRD results.

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