This article has been accepted for publication in a future issue of this journal, but has not been fully edited. Content may change prior to final publication. Citation information: DOI 10.1109/TNB.2015.2461444, IEEE Transactions on NanoBioscience

A High Sensitivity Impedimetric Biosensor Using the Tannin From *Quercus macrolepis* as Biorecognition Element for Heavy Metals Detection

N. Khedimallah, A. Zazoua*, A. Sbartai, N. Jaffrezic-Renault

***Abstract*— A new poly(vinylchloride) (PVC) membrane electrode based on tannin from the bark of *Quercus macrolepis* (acorn) as the ionophore was prepared and modified onto the surface of a gold electrode. The electrochemical impedance spectroscopy (EIS) technique was used to study the sensitivity of the electrode that was modified with a thin layer of polymeric biomembrane, in order to detect heavy metals ions in solution. The device shows a good sensitivity for Zn^{2+}, Ni^{2+} ions and a little less for Cd^{2+}. The electrode indicates a good linear response for the three metals over a wide concentration range from 1.0×10^{-9} to 1.0×10^{-4} M, with a detection limit of 1.0×10^{-9} M.**

***Index Terms*— Tannin, heavy metals, sensor, impedance spectroscopy, pollution.**

I. Introduction

Whether accidental or intentional, the pollution of waters and soils by some chemical products whose origin may be industrial (heavy metals, dyes…) or agricultural rise to worldwide particular interest [1-3]. Beyond a certain concentration, heavy metals do turn into highly toxic species [4]. They have the ability to concentrate along the food chain and pile up inside certain organs of the body, inducing a number of health problems, especially in long run [5]. For thirty years now, a handful of doctors and researchers scattered about in various parts of the world have begun to find a very clear causal link between the chronic exposure to toxic heavy metals and many diseases. Since then, scientific researchers cannot but confirm this thesis [6, 7]. While heavy metal pollution proves to be a serious health problem, the most generally used methods of analysis at the present time are complex, heavy and expensive. This has prompted researchers to come up with a simple analytical tool that consist of an electrochemical sensor composed of a sensitive membrane with a selective ionophore and a transducer which converts the (bio) chemical interaction between the metal ion and the ionophore into a measurable and quantifiable electric signal [8-12].

Electrochemical sensors as an alternative to the spectroscopic techniques have been accepted because they proved to be effective for the detection of trace metal ions because of their excellent sensitivity, low cost, portability and above all their very short time for analysis.

The use of gold electrodes as transducers has been the subject of several studies [13-17]. Thus, a number of researchers have immobilized membranes or biomembranes on this kind of electrode in order to form a sensor. Enzymatic membranes have been developed for the detection of heavy metals based on the inhibition of the enzyme [18-20]; but this kind of biosensor is very fragile and very sensitive with respect to several parameters such as temperature and pH. Other features, including chemical membranes, have come out [21-24]. In this kind of sensors, selectivity and detection limit remain below the mark, which considerably restricts their use.

In this piece of work, we have developed a sensor by modifying the surface of a gold electrode with a polymer membrane composed of poly(vinylchloride) as a matrix containing tannin extracted directly from the shell of the *Quercus macrolepis*, the fruit of the oak tree from the southwest of the Mediterranean. Naturally, we used a conventional method of extraction, maceration. Therefore, the device was formed as an electrochemical sensor for the detection of Zn^{2+}, Ni^{2+} and Cd^{2+} in trace levels. The electrochemical characterization of our developed devices was carried-out by impedance spectroscopy and cyclic voltammetry.

The use of condensed tannins as ligand for the functionalization of gold electrodes constitutes a great innovation. As a matter of fact, these natural substances have been the subject of several scientific works in the field of water treatment using them as adsorbents for the removal of heavy metals in aquatic systems [25-29]. Tannins have changeable structures that invariably include a phenolic side. We have used condensed tannins which are polyphenols of the

N. Khedimallah is with Laboratoire LMEPA, Université de Jijel, BP 98, Ouled Aissa 18000, Algérie (khedimallahn@yahoo.com).

*A. Zazoua is with Laboratoire LMEPA, Université de Jijel, BP 98, Ouled Aissa 18000, Algérie (azazoua@yahoo.fr).

A.Sbartai is with Université de Lyon-ISA-UMR 5180 CNRS-Université Claude Bernard Lyon 1, 69622 Villeurbanne Cedex, France. (asbartai@hotmail.com).

N. Jaffrezic-Renault is with Université de Lyon-ISA-UMR 5180 CNRS-Université Claude Bernard Lyon 1, 69622 Villeurbanne Cedex, France. (nicole.jaffrezic@univ-lyon1.fr).

This article has been accepted for publication in a future issue of this journal, but has not been fully edited. Content may change prior to final publication. Citation information: DOI 10.1109/TNB.2015.2461444, IEEE Transactions on NanoBioscience

flavonoid family and whose chemical structure is based on a heterocyclic system. In their chemical structure, they show adjacent functional groups of the polyhydroxyphenyl, which gives them a very high affinity with metal ions.

II. Materials and Methods

A. Reagents

Tannins, the sensing molecules used in this work, were extracted via the Laboratory of Chemical Engineering of Jijel University in Algeria. The barks of acorn were shade dried and coarsely powdered. To extract the condensed tannins from the fruit (the acorn) of the oak tree by maceration, we have opted for the standard protocol but with some modifications: 10 to 30 g of shell of the acorn powder were macerated at room temperature for 2.5 hours (twice) with 100 mL of an aqueous solution of the following solvents: ethanol, acetone, 70% methanol V/V and water. After filtration over a muslin cloth, the filtrates were centrifuged for 20 minutes at 4000 rpm/min at room temperature, filtered through filter paper n°1 and stored at 4°C for later use.

All other reagents of analytical grade were obtained from Sigma-Aldrich and were used without further purification.

B. Sensor design and membrane immobilization

The transducers used are gold macroelctrodes fabricated using standard silicon technologies. They are deposited by evaporation under vacuum onto Si/SiO2 substrates by using a 30 nm titanium bonding layer. These gold electrodes are provided by the Laboratory for Analysis and Architecture of Systems (LAAS), Toulouse, France. A resin layer is deposited on the gold surface to protect it during cutting and storage. When they are attached via an O-ring on the electrochemical cell, these electrodes active surface is about 0.19 cm2.

Before their use, the gold electrodes are cleaned in two steps. In a first step, the electrodes are dipped in acetone for 10 minutes before they are dried up under a nitrogen stream. An attack by a "piranha" solution (a solution composed of 2/3 of concentrated sulphuric acid and 1/3 of hydrogen peroxide) is carried out in a second step for one minute. The electrodes are rinsed with ultra-pure water and then with ethanol before being dried up under a nitrogen stream.

The thin film representing the sensitive part of the sensor was prepared by using a suspension of polyvinyl chloride (polymer), dinonyl phthalate (plasticizer) and tannin ionophore in the following proportions: 7 w/w%; 30 w/w%; 62 w/w%, diluted in tetrahydrofuran (THF), respectively. The membrane was obtained by depositing 5 µL of the aforementioned PVC–DNP/ionophore suspension onto the gold electrode using a spin-coater with the following parameters: rpm = 2500, rpm/s = 2000, t = 30 s. Afterwards, the surface was dried in air for 15 h.

C. Electrochemical measurements

The PVC-tannins electrode has been characterized by two electrochemical methods, to wit: impedance spectroscopy and cyclic voltammetry.

Impedance measurement and cyclic voltammetry are based on the experimental set in fig.1. It consists of a potentiostat, an impedance analyser, a computer for controlling the instruments, acquiring and processing data and of an electrochemical cell consisting of three electrodes:

- A working electrode made of gold and modified by the PVC-tannins membrane, upon which are examined the various electrochemical processes to be explored.
- A saturated calomel reference electrode whose potential is constant and known, which allows us to control the working electrode potential.
- A platinium auxiliary electrode referred to also as counter-electrode which helps close the electric circuit in the electrochemical cell.

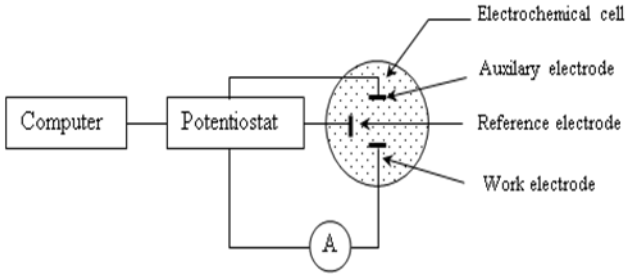

Fig. 1. Schema of the experimental setup for electrochemical measurements

All the electrochemical experiments were performed with a "VoltaLab 40" impedance analyser (Radiometer Analytical SA). The electrochemical cell is placed in a Faraday cage in order to reduce the influence of electromagnetic waves. For the impedance spectroscopy measurements, this device is used both to generate the sinusoidal signal with the desired amplitude and frequency, and to extract the real as well as the imaginary parts of the impedance of the system under study. The impedance measurements were performed in the frequency range from 100 kHz to 100 mHz. The potential polarization used for these measurements was optimized and a 0.05 M PBS buffer solution at pH 7.2 was used as the electrolyte. The impedance analyser is controlled by a computer with the help of the software "Volta Master 4.0".

The cyclic voltammetry measurements were performed using the same equipment. The potential was cycled from −400 to 600 mV (vs. the reference electrode) with a scan speed of approximately 25 mV/s in a PBS buffer solution containing 2 mM K3Fe(CN)6 and K4Fe(CN)6, respectively. All electrochemical measurements were carried out inside a Faraday cage.

III. Results and Discussion

A. Electrochemical characterization of Au/PVC-tannin/electrolyte interface

Cyclic voltammetry experiments can be used to characterize the PVC-tannin thin layer deposited on the gold surface. The cyclic voltammograms before and after the functionalization of the gold electrode is shown in fig. 2.

This article has been accepted for publication in a future issue of this journal, but has not been fully edited. Content may change prior to final publication. Citation information: DOI 10.1109/TNB.2015.2461444, IEEE Transactions on NanoBioscience

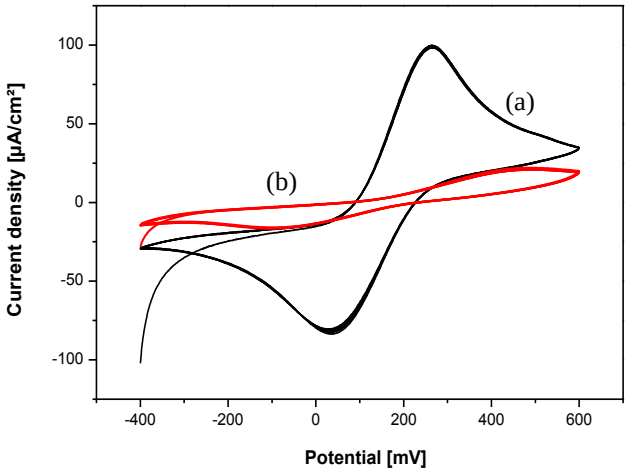

Fig. 2. The cyclic voltammograms of a 2 mM equimolar mixture of K3Fe(CN)6 and K4Fe(CN)6 recorded in phosphate buffer saline pH 7.2 at a bare gold electrode (a) and at the same electrode after Tannins/PVC deposition (b). Scan rate: 25 mV s−1 vs SCE.

The oxidation peak and that of the reduction of the redox couple are visible before the deposition of the polymeric membrane. The potential difference between the two peaks is $\Delta E = 210$ mV and the peaks have the same height, suggesting a reversible process indicating the cleanliness of the surface due to the efficiency of the electrode cleaning treatment. After the deposition, a reduction in the height of the peaks is noticed; the potential difference changes to $\Delta E = 500$ mV, which can be attributed to the decrease in the electron transfer through the polymeric membrane which means that electrons cannot be freely transferred to the electrode.

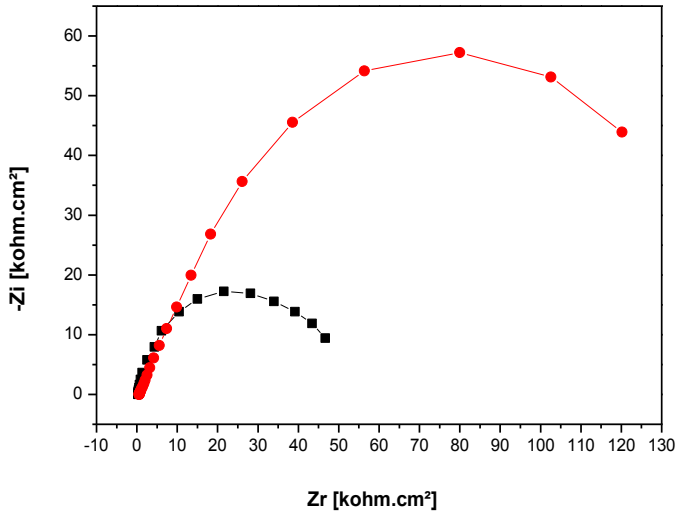

Fig. 3. Nyquist plots (a) of the complex impedance spectra of the gold electrode before (■) and after (●) Tannins/PVC deposition. Zim/kΩ represents the imaginary component and Zre/kΩ the real component of the complex impedance Z(ω).

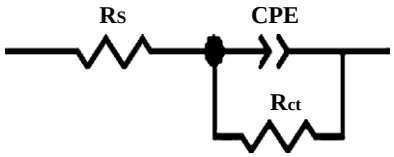

Fig. 4. Bare electrode data modelled with the electrical equivalent. Rs is the ohmic resistance of the electrolyte solution, Rct the charge transfer resistance and CPE the constant phase element.

For the study of the sensor sensitivity, optimizing the polarization is important. The Warburg diffusion impedance has to be reduced in order to increase recognition. In actual fact, polarization varies with the membrane used as recognition layer.

In our study, different values of the potential were applied on a wide range of frequencies. For a polarization equal to -400 mV/ECS, Warburg impedance was reduced to a minimum and the diameter of the semicircle shortened, which shows the effect of the charge transfer reduction, thus giving us the possibility to observe the influence of the sensitivity at the ion exchange.

The Nyquist diagrams obtained in the complex plane for the bare electrode and the one functionalized by the PVC-Tannins membrane are shown in fig. 3. A strong increase in impedance is noticed after the electrode functionalization phase, which confirms the fact that the transfer of electrons to the electrode has been slowed down by the addition of membrane.

The Nyquist Z spectra are adjusted by the Zplot/Zview software (Scribner Associates Inc.) which helps setting up the equivalent electric circuit. The best fit was obtained with a correlation coefficient $\chi 2 = 10^{-3}$ by using the equivalent circuit shown in fig. 4.

The resistance of the electrolyte, the charge transfer resistance and the constant phase element are represented Rs, Rct and CPE respectively. The impedance spectroscopy theory shows that the capacity of the double layer for an electrochemical cell behaves as a constant phase element, CPE. Interfacial irregularities (porosity, geometry, roughness, diffusion,…) are taken into account by this constant phase. It is defined by a CPE capacity and αCPE number which can take -1, 0, 0.5 and 1 values corresponding respectively to an inductance, pure impedance, the Warburg terms or capacitance. Due to surface heterogeneity, the impedance Z(ω) of such a non-ideal layer can be expressed as $Z(\omega)=CPE^{-1}(j\omega)^{-n}$, where ω is a circular frequency and n parameter varies from 0 to 1.When n is close to 0, CPE is essentially a resistor. If n=1, the CPE is a pure capacitor and the electrode is considered as ideal.

In order to test the sensitivity of our chemical sensor, different concentrations of Cd(II), Zn(II) and Ni(II) were added to the buffer solution. Each metal was separately studied. The impedance measurements were carried out under optimised conditions.

This article has been accepted for publication in a future issue of this journal, but has not been fully edited. Content may change prior to final publication. Citation information: DOI 10.1109/TNB.2015.2461444, IEEE Transactions on NanoBioscience

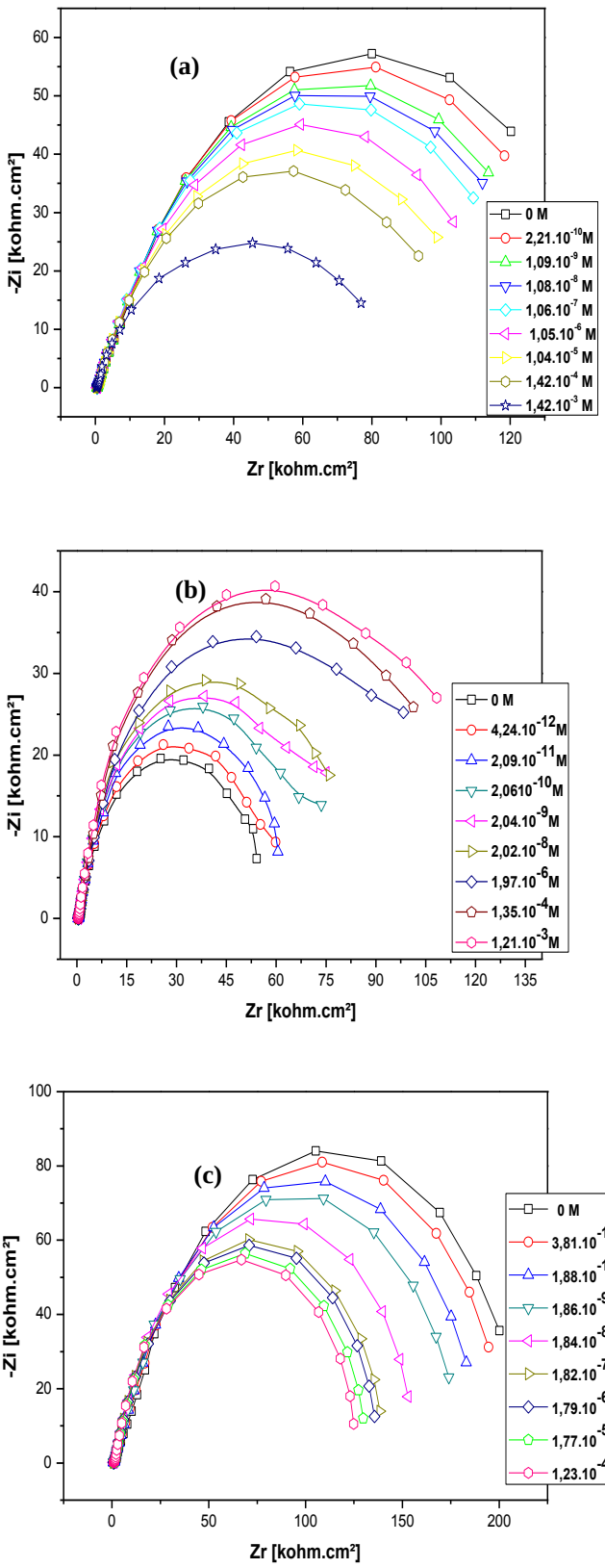

Fig. 5. Nyquist diagram (−Zim vs. Zr) at −0,4 V versus SCE in PBS solution pH 7.3 obtained for the impedance measurements on the tannin biosensor under various concentrations of (a) Cd(II), (b) Ni (II) and (c) Zn (II) concentrations. Spectra were obtained between 1 mHz and 100 kHz. Amplitude of alternative voltage: 10 mV

As shown in fig. 5, the diameter of the impedance diagrams decreases with increasing Cd(II), Zn(II) and Ni(II) concentration. This was especially observed at low frequency. This phenomenon can be explained by the biochemical bonds of the metals with the tannins membrane which are expected to affect interfacial properties of the working electrode, inducing variations of resistance and capacitance of the equivalent circuit. Thus, a variation of the dielectric properties of tannin-PVC films with the affinity of such sensing molecules towards Cd(II), Zn(II), and Ni(II) ions was detected.

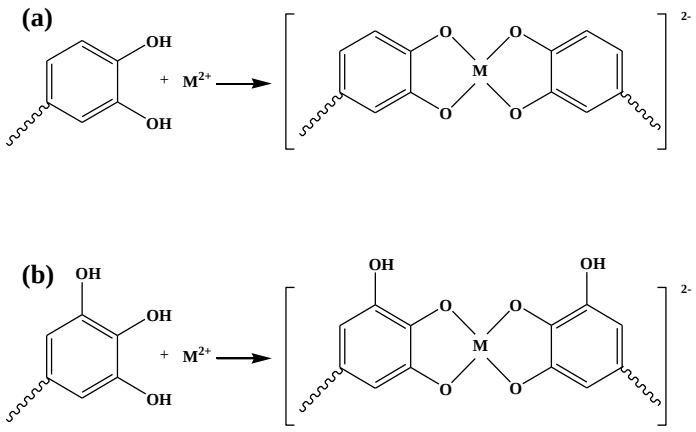

Fig. 6. (a): Cathecol group-complexation. (b): Pyrogallol group complexation.

The reactional mechanism could be an ion exchange reaction or one of complexation between the metal ions and the phenolic groups (pyrogallol or catechol groups) which constitute the polyphenols. Hence, this reaction can be represented in two ways (fig. 6). Several authors [30-34] confirmed that the interactions between metal ions and the polyphenols are possible through the adjacent hydroxyl groups of tannin. The studies revealed an interaction between orthohydroxyl groups present in their structures and the metal ions. Metal cations displace the protons on the adjacent phenolic hydroxyl groups forming a chelate. The oxygen on the phenol group is considered as a strong base because of the presence of its pairs of nonbonding electrons. With these pairs of electrons, the oxygen base can form coordination complexes with chemical entities lacking electrons, such as metal ions. In the case of our divalent cations, the metal ion moves the two protons away from the adjacent hydroxyl groups, which could add two pairs of electrons to the metal ions and, in so doing, form the chelate compound.

To obtain the calibration curve of the three metals-ion sensors, we have plotted the variations of Rct vs the Cd (II), Zn (II) and Ni (II) concentration, respectively. Fig. 7 shows the comparison between the calibrations curves of the three metals tested.

This article has been accepted for publication in a future issue of this journal, but has not been fully edited. Content may change prior to final publication. Citation information: DOI 10.1109/TNB.2015.2461444, IEEE Transactions on NanoBioscience

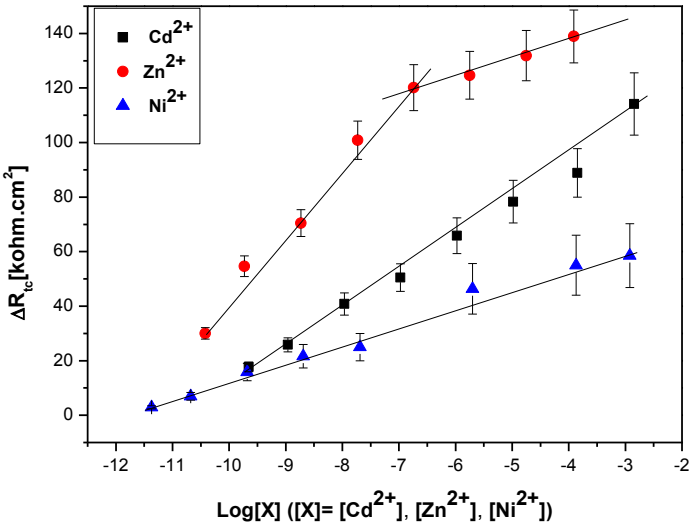

Fig. 7. Calibration curves describing the variation of charge transfer resistance Rtc against Zn (II), Cd (II) and Ni (II) concentration.

Here, a good response for the three metals detection was shown, especially for Zn2+. We notice that the response of the Zn2+present two levels. Indeed, there is a competitiveness and rapid response towards tannins initially. This phenomenon can be explained by the relatively high electronegativity and its relatively small ionic radius compared to the other two metals in this case the cadmium and the nickel. There is therefore a relatively high selectivity of zinc with tannins of *Quercus macrolepis* which promotes a rapid chelating and complexing of metal on the biomembrane. In a second step, a beginning saturation of the active site is noticeable. This is what has generated a decrease of the response and hence the onset the second bearing. The calibration curves are linear for the two metal ions and in a linear dynamic range for Cd (II) and Ni (II) (linear up to 10-3 M). Moreover, the response of Zn (II) shows a large linear range. The observed response was linear with a slope of 24.1 mV per decade and it was quasi Nernstian for bivalent anions for concentrations between 10−9 to 10−3 M. The linear model was validated for the three metals, and the detection limits obtained for these three metals were, around 10-9 M. The sensitivity is an important parameter for low detection limits. Typically, a higher sensitivity will result in a lower limit of detection. The sensitivities calculated from the calibration curves were in decreasing order from Zn (II), Cd (II), and Pb (II) respectively. Therefore, the reaction mechanism of the three metals overlooked to the biomembrane can be influenced by other parameters such as electronegativity and ionic radius [27].

Comparing our results with those previously published about heavy metals electrodes detection shows a very low detection limits obtained with our device functionalized with the tannic membrane. The results in Table 1 confirm clearly the synergy effect when we combine the physical and chemical properties of the biomembrane-based tannin from *Quercus macrolepis* with the electrochemical characteristics of the transducer electrode.

TABLE I
COMPARISONS WITH THE REPORTED WORKS ON HEAVY METAL DETECTIONS

Metal ion	Interface material	Limite of detection LOD	Ref
Zn	Polyaniline/HRP	36.8 nM	[35]
Zn	Diphenilbenzidine/CTAB	0.1 µM	[36]
Zn	HRP/Maize tassel-Multiwalled carbon nanotube	7.5 µg.L^{-1}	[37]
Zn	PVC/Tannins from *Quercus macrolepis*	0.2 nM	Our result
Cd	Calix[4]arene	10^{-6} M	[38]
Cd	Screen printed electrodes	500 nM	[39]
Cd	Multiwalled carbon nanotubes with cyclic dipeptide	2.74.10^{-8} M	[40]
Cd	PVC/Tannins from *Quercus macrolepis*	1.1 nM	Our result
Ni	BDD	33 nM	[41]
Ni	BDD	6.8 nM	[42]
Ni	PVC/Tannins from *Quercus macrolepis*	0.8 nM	Our result

IV. CONCLUSION

A gold electrode functionalized with PVC-tannin biomembrane was used for heavy metals determination, namely, Cd^{2+}, Zn^{2+} and Ni^{2+} by using electrochemical impedance spectroscopy technique. Chemical modification of the electrode surface was a very large contribution to increase the detection limit and sensitivity of the developed device. The proposed analytical technique was rapid, simple, and low-cost in comparison with other analytical methods used for the detection of heavy metals. This study has shown that a good sensitivity and low detection limit of 10^{-9} M were obtained for the three heavy metals. The tannin biomembrane displayed a high affinity and high response sensitivity to Zn (II). It was shown that impedimetric responses was linear for Cd^{2+}, Zn^{2+} and Ni^{2+} between concentrations from 10^{-9} to 10^{-3} M. This demonstrates the great potential of these biosensors for quantitative detection of heavy metals. Therefore, gold electrodes functionalized with a biomembrane which is environmental friendly can be strongly recommended as an electrochemical sensor for heavy metals in wastewater due to its rapidity, simplicity, and accuracy.

ACKNOWLEDGMENT

This work was supported by the CNEPRU N° J0101720140122.

REFERENCES

[1] K. Karlsson, M. Viklander, L. Scholes, M. Revitt, "Heavy metal concentrations and toxicity in water and sediment from stormwater ponds and sedimentation tanks", *Journal of Hazardous Materials*, vol. 178, pp. 612-618, 2010.

[2] S. Das, R. Raj, N. Mangwani, H. R. Dash, J. Chakraborty, "Heavy Metals and Hydrocarbons: Adverse Effects and Mechanism of Toxicity", *Microbial Biodegradation and Bioremediation*, pp. 23-54 2014.

[3] P. Madoni, M. G. Romeo, "Acute toxicity of heavy metals towards freshwater ciliated protists". *Environmental Pollution*, vol. 141, pp. 1-7, 2006.

[4] J. Chen, Y. Jiang, C. Xu, L. Yu, D. Sun, L. Xu, F. Hu, H. Li, "Comparison of two mathematical prediction models in assessing the toxicity of heavy metal mixtures to the feeding of the nematode Caenorhabditis elegans", *Ecotoxicology and Environmental Safety*, vol. 94, pp. 73-79, 2013.

[5] M.K. Türkdoğan, F. Kilicel, K. Kara, I. Tuncer, I. Uygan, "Heavy metals in soil, vegetables and fruits in the endemic upper gastrointestinal cancer region of Turkey", *Environmental Toxicology and Pharmacology*, vol. 13, pp. 175-179, 2003.

[6] K. Khan, H. Khan, Y. Lu, I. Ihsanullah, J. Nawab, S. Khan, N. S. Shah, I. Shamshad, A. Maryam, "Evaluation of toxicological risk of foodstuffs contaminated with heavy metals in Swat, Pakistan", *Ecotoxicology and Environmental Safety*, vol. 108, pp. 224-232, 2014.

[7] B. Zhong, H. Mao, Q. Fan, Y. Liu, Y. Hu, Y. Mi, F. Wu, C. Hu, "siRNA-mediated knockdown of CiGRP78 gene expression leads cell susceptibility to heavy metal cytotoxicity", *Gene*, vol. 552, pp. 219-224, 2014.

[8] A. Zazoua, R. Kherrat, M.H. Samar, A. Errachid, N. Jaffrezic-Renault, F. Bessueille, D. Léonard, "Characterization of TBP containing polysiloxane membrane/insulator/semiconductor structures for hexavalent chromium detection", *Materials Science and Engineering: C*, vol. 28, pp. 1014-1019, 2008.

[9] A. Zazoua, I. Kazane, N. Khedimallah, C. Dernane, A. Errachid, N. Jaffrezic-Renault,"Evidence of ammonium ion-exchange properties of natural bentonite and application to ammonium detection", *Materials Science and Engineering: C*, vol. 33, pp. 5084-5089, 2013.

[10] A. Sbartai, P. Namour, "Electrochemical Boron-Doped Diamond Film Microcells Micromachined with Femtosecond Laser: Application to the Determination of Water Framework Directive Metals", *Anal. Chem*, vol. 84, pp. 4805−4811, 2012.

[11] H. Lei, W. Li, X. Tan, "Encapsulation of ionic polymer-metal composite (IPMC) sensors with thick parylene: Fabrication process and characterization results", *Sensors and Actuators A: Physical*, vol. 217 pp. 1-12, 2014.

[12] C. Dernane, A. Zazoua, I. Kazane, N. Jaffrezic-Renault, "Cadmium-sensitive electrode based on tetracetone derivatives of p-tert-butylcalix[8]arene", *Materials Science and Engineering: C*, vol 33, pp. 3638-3643, 2013.

[13] G-X. Zhong, W-X. Zhang, Y-M. Sun, Y-Q. Wei, Y. Lei, H-P. Peng, A-L. Liu, Y-Z. Chen, X-H. Lin, "A nonenzymatic amperometric glucose sensor based on three dimensional nanostructure gold electrode", Sensors and Actuators B: Chemical, vol. 212, pp. 72-77, 2015.

[14] N. Xia, F. Ma, F. Zhao, Q. He, J. Du, S. Li, J. Chen, L. Liu, " Comparing the performances of electrochemical sensors using p-aminophenol redox cycling by different reductants on gold electrodes modified with self-assembled monolayers", *Electrochimica Acta*, vol. 109, pp. 348-354, 2013.

[15] A. Ferrario, M. Scaramuzza, E. Pasqualotto, A. De Toni, A. Paccagnella, "Development of a Disposable Gold Electrodes-Based Sensor for Electrochemical Measurements of cDNA Hybridization", *Procedia Chemistry*, vol. 6, , pp. 36-45, 2012.

[16] K. Rovina, S. Siddiquee, N. K. Wong, "Development of melamine sensor based on ionic liquid/nanoparticles/chitosan with modified gold electrode for determination of melamine in milk product, "*Sensing and Bio-Sensing Research*", vol. 4, pp. 16-22, 2015.

[17] M. Shamsipur, M. Asgari, M. Ghannadi Maragheh, D. Matt, "Impedimetric sensing of cesium ion based on a thiacalix[4]arene self-assembled gold electrode", *Sensors and Actuators B: Chemical*, vol. 209, pp. 9-14, 2015.

[18] N. Bouyahia, M. L. Hamlaoui, Hnaien M, F. Lagarde, N. Jaffrezic-Renault, "Impedance spectroscopy and conductometric biosensing for probing catalase reaction with cyanide as ligand and inhibitor", *Bioelectrochemistry*, vol. 80, pp. 155-161, 2011.

[19] A.Zazoua, C. Dernane, I. Kazane, M. Belghobsi, A. Errachid, N. Jaffrezic-Renault, "Gold electrode functionalized with catalase for impedimetric detection of nitrite", *Sensor Letters*, vol. 9, N° 6, pp. 2283-2286, 2011.

[20] M. E. Ghica, R. C. Carvalho, A. Amine, C. M.A. Brett," Glucose oxidase enzyme inhibition sensors for heavy metals at carbon film electrodes modified with cobalt or copper hexacyanoferrate", *Sensors and Actuators B: Chemical*, vol. 178, pp. 270-278, 2013.

[21] Z.Q. Tou, T.W. Koh, C.C. Chan, "Poly(vinyl alcohol) hydrogel based fiber interferometer sensor for heavy metal cations", *Sensors and Actuators B: Chemical*, vol. 202, pp. 185-193, 2014.

[22] P. Niu, C. Fernández-Sánchez, M. Gich, C. Ayora, A. Roig, "Electroanalytical Assessment of Heavy Metals in Waters with Bismuth Nanoparticle-Porous Carbon Paste Electrodes", *Electrochimica Acta*, vol. 165, pp. 155-161, 2015.

[23] Manju Bhargavi Gumpu, Swaminathan Sethuraman, Uma Maheswari Krishnan, John Bosco Balaguru Rayappan, "A review on detection of heavy metal ions in water – An electrochemical approach", *Sensors and Actuators B: Chemical*, vol. 213, pp. 515-533, 2015.

[24] Wooseok Jung, Am Jang, Paul L. Bishop, Chong H. Ahn, "A polymer lab chip sensor with microfabricated planar silver electrode for continuous and on-site heavy metal measurement", *Sensors and Actuators B: Chemical*, vol. 155, n° 1, pp. 145-153, 2011.

[25] V.C.T. Costodes, H. Fauduet, C. Porte, A. Delacroix "Removal of Cd(II) and Pb(II) ions, from aqueous solutions, by adsorption onto sawdust of Pinus sylvestris", *J. Hazard. Mater*. vol. 105, pp. 121–142, 2003.

[26] M. Ozacar and A. Singel, "Adsorption of metal complex dyes from aqueous solutions by pine sawdust", *Bioresour. Technol*, vol. 96, pp. 791-795, 2005.

[27] I. A. Sengil, M. Ozacar, "Competitive biosorption of Pb2+, Cu2+ and Zn2+ ions from aqueous solutions onto valonia tannin resin", *J. Haz. Mat*, Vol. 166, pp. 1488–1494, 2009.

[28] S. Nirmala, H.N. Ahamed, V. Ravichandiran, "Comparative in vitro study on the free radical scavenging capacity of tannin and biflavone fraction from Ficus racemosa Linn and Araucaria bidwilli Hook", *International Journal of ChemTech Research*, vol 3, pp. 1440-1445, 2011.

[29] P. Ramakul, Y. Yanachawakul, N. Leepipatpiboon, N. Sunsandee, "Biosorption of palladum (II) and platinium (IV) from aqueous solution using tannin from Indian almond (Terminalia catappa L.) leaf biomass: Kenetic and equilibrium studies", Chemical Engineering Journal, vol. 193, pp. 102-111, 2012.

[30] S.G. Chung, J.C. Ryu, M.K. Song, B. An, S.B. Kim, S.H. Lee, J.W. Choi, "Modified composites based on mesostructured iron oxyhydroxide and synthetic minerals: A potential material for the treatment of various toxic heavy metals and its toxicity", *Journal of Hazardous Materials*, vol. 267, pp. 161-168, 2014.

[31] Y. Nakano, K. Takeshita, T. Tsutsumi, "Adsorption mechanism of hexavalent chromium by redox within condensed tannin gel", *Water Res*, vol. 35, pp. 496-500, 2001.

[32] A. Nakajimaa, Y. Babab, "Mechanism of hexavalent chromium adsorption by persimmon tannin gel", *Water Res*, vol. 38, pp. 2859-2864, 2004.

[33] J. Beltran and J.S. Martin, "Removing heavy metals from polluted surface water with a tannin-based floculant agent", *Journal of Hazardous Materials*, vol. 165, pp. 1215-1218, 2009.

[34] M. Yurtsever, I.A. Sengil, "Biosorption of Pb(II) ions by modified quebracho tannin resin", *Journal of Hazardous Materials*, vol. 163, pp. 58-64, 2009.

[35] P.N. Nomngongo, J.C. Ngila, T.A.M. Msagati, "Indirect amperometric determination of selected heavy metals based on horseradish peroxidase modified electrodes", *Biosens. Emerging Mater. Appl.*, pp. 569–588, 2008.

[36] D.K. Das, "Electrochemical determination of Zn^{2+} ion using diphenylamine/single walled carbon nanotube/cetyltrimethylammonium bromide modified glassy carbon electrode", *J. Surf. Sci. Technol*., vol. 24, pp. 149–162, 2008.

[37] M. Moyo, "Horseradish peroxidase biosensor to detect zinc ions in aqueous solutions", *J. Appl. Biosens*., vol. 20, pp. 1–7, 2014.

[38] K. Morakchi, A. Hamel, R. Kherrat, "Electrochemical sensors for the detection of cadmium (II) based on calix [4] arene", *Desalin. Water Treat*., vol. 46, pp. 168–170, 2012.

[39] O. Krystofova, L. Trnkova, V. Adam, J. Zehnalek, J. Hubalek, P. Babula, R. Kizek, "Electrochemical microsensors for the detection of cadmium(II) and lead(II) ions in plants", *Sensors*, vol. 10, pp. 5308–5328, 2010.

[40] N.A. Rahman, N.A. Yusof, N. Amirah, M. Maamor, S. Mariam, M. Noor, "Development of electrochemical sensor for simultaneous determination of Cd(II) and Hg(II) ion by exploiting newly synthesized cyclic dipeptide", *Int. J. Electrochem. Sci.*, vol. 7, pp. 186–196, 2012.

[41] Y. R. Zhang, S. Yoshihara, "Cathodic stripping voltammetry of nickel on boron-doped diamond", *J. Electroanal. Chem*., vol. 573, pp. 327−331, 2004.

[42] A. Sbartai, , P. Namour, "Electrochemical Boron-Doped Diamond Film Microcells Micromachined with Femtosecond Laser: Application to the Determination of Water Framework Directive Metals", *Anal. Chem.* Vol. 84, pp. 4805−4811, 2012.

First nawal khedimallah is a doctoral student in chemistry department at jijel university, algeria. the current research interest is electrochemical sensor.

Second Dr Ali Zazoua was born in Jijel, Algeria. He is graduated in Chemical engineerieng from the University Badji Mokhtar, Annaba and received a PhD degree (2008) and HDR, Habilitation to Direct Research, Process Engineering, from Annaba University (Algeria) in 2010. He is working as Senior Lecturer and senior research scientists in Jijel University, Algeria. The current research interests are electroanalysis and electrochemical sensors and biosensors

Third Dr Amel Sbartai was born in Algeria made her master studies in University Badji Mokhtar Annaba, she then started his thesis which consisted in the detection of heavy metals by chemical sensors at the University Claude Bernard Lyon1, where she obtained her doctorate diploma in 2014, she continues her research now at the Institute of vision, she works on the electrochemical caracteristaion of retinal implants.

Fourth Dr Nicole Jaffrezic-Renault was received her engineering degree from the Ecole Nationale Supérieure de Chimie, Paris, in 1971 and the Doctorat d'Etat és Sciences Physiques from the University of Paris in 1976. As Director of Research at the Centre National de la Recherche Scientifique, past president of the chemical micro sensor club (CMC2), president of the Analytical Division of the French Chemical Society, her research activities in the Institute of Analytical Sciences, include conception and design of (bio)chemical sensors and their integration in microsystems. She coordinates several European and national projects for the development of microsystems for biomedical and environmental monitoring and for food safety. She published more than 500 papers with more than 7900 citations (H index: 40).