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Rabiaa Helim, Ali Zazoua, Nicole Jaffrezic-Renault, Hafsa Korri-Yousoufi. Label free electrochemical sensors for Pb(II) detection based on hemicellulose extracted from *Opuntia Ficus Indica cactus*. *Talanta*, 2023, 265, pp.124784. 10.1016/j.talanta.2023.124784 . hal-04299636

**HAL Id: hal-04299636**

**<https://hal.science/hal-04299636v1>**

Submitted on 1 Dec 2023

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# Label free Electrochemical sensors for Pb(II) detection based on hemicellulose extracted from *Opuntia Ficus Indica* cactus

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## Abstract

We aim to develop an electrochemical sensor for a divalent metal ion (lead II), a highly toxic water contaminant. We explore a sensor formed with a hemicellulose polysaccharide extracted from the *Opuntia ficus indica* cactus associated with agarose as a sensitive layer deposited on a gold electrode. This sensor combines the functional groups of hemicellulose that could form a complex with metal ions and agarose with gelling properties to form a stable membrane. The sensor demonstrated a loading ability of Pb<sup>2+</sup>, with higher affinity than other metal ions such as Hg<sup>2+</sup>, Ni<sup>2+</sup>, and Cu<sup>2+</sup>, thanks to the chemical structure of hemicellulose. The detection was measured by square wave voltammetry based on a well-defined redox peak of the metal ions. The sensor shows high sensitivity towards Pb<sup>2+</sup> with a detection limit of 1.3 fM. The application in the river and the sea with the standard addition method of sensing a lead was studied.

**Keywords:** Heavy metals, Hemicellulose, *Opuntia ficus indica*, Polysaccharide, Sensors.

## Introduction

Water pollution from toxic metals and organic compounds remains a severe environmental and public problem [1]. In the face of increasingly stringent regulations, water pollution has become a significant concern and a priority for most industrial sectors [2]. Heavy metal ions are often found in the environment because of their wide use. They are common contaminants in wastewater and rivers. Many are known to be predominantly toxic or carcinogenic and adversely affect vegetation, animals, and humans, even at low concentrations [3]. They primarily penetrate the body by inhalation and ingestion and affect many organic systems. For all metals, the maximum tolerated concentration established by the World Health Organization (WHO) ranges from 482 to 24 nM (1 to 50 µg/L) in drinking water [4]. Lead ion ( $\text{Pb}^{2+}$ ) is one of the most toxic heavy metal ions that can induce various serious diseases, including neurological disorders, cardiovascular disease, and memory lapses. Considering its severe toxicity, World Health Organization has set the highest allowable limit for  $\text{Pb}^{2+}$  in drinking water at 4.82 nM (10µg/L) [5]. Consequently, it is essential to find a sensitive, fast, and simple analytical method to determine these metals [6] accurately. Spectrometric techniques such as Flame Atomic Absorption Spectrometry (ETAAS and FAAS), Inductively Coupled Plasma techniques (ICP-OES and ICP-MS), and Atomic Absorption Spectroscopy (AAS) remain the most widely used techniques for metal ions determination in different samples [5]. While these techniques can provide high selectivity, they also have some drawbacks, including operation complexity, time and reagents consumption, high cost, and, most importantly, a need for qualified personnel to use them [7, 8]. Developing sensors or biosensors is the most appropriate solution to this problem. Indeed, sensors and biosensors should be able to continuously give a sensitive, selective, and rapid response to the presence of specific compounds directly in a complex mixture without the need for sample treatment. Sensors have a significant advantage over conventional analytical methods, which require various sample treatments before measurement. In addition, sensors and biosensors have many unique features, such as compact size, ease of use, and the ability to provide continuous signals in real-time [9]. Recently, considerable efforts have been made in the electrochemical biosensors field to detect metal ions based on various biomolecules. Thus, these bio-receptors are the critical element in the success of ionosensors. Various biomolecules are designed to target metal ions. For example, we can cite whole cells [10, 11], DNA-zymes and DNA aptamers [12-14], and recent reviews have been published in this field [15, 16]. Aptamer-based sensors have been considered an innovative tool in metal monitoring due to their stable interaction, weak detection limit, and high selectivity [3, 17]. While DNA-zyme based biosensors are also capable of specific detection of some metal ions due to their catalytic reaction and cleavage of DNA [18, 19]. However, organic ionophores are the subject of several studies, and calix[4]arene derivatives are some of the researched selective ionophore for lead [20-22].

The evolution in the electrochemical ionosensors field has also widened by including materials and nanomaterials. Introducing carbon nanotubes, graphene, and metal oxide nanomaterials, whether or not they are bound to polymeric matrices [23-27], improves the electrochemical response and the sensitivity. Ionosensors have used a wide range of polymers, and the commonly encountered are conductive polymers such as polyacetylene (PAC), polyaniline (PANI), polypyrrole (PPy), poly (p-phenylene) (PPP), polythiophene (PTh) and poly (3,4-ethylenedioxythiophene) (PEDOT) [28].

Others approach to designing ionosensors are based on the use of natural polymers. Indeed, biological resources can detect metal ions efficiently [11]. Natural polymers or biopolymers, in particular polysaccharides, can provide sensing materials for metal ions due to their particular structure, physicochemical characteristics, chemical stability, high reactivity, and excellent selectivity. The presence of chemically reactive groups (hydroxyl, carboxyl, or amine functions) in these biopolymers provides coordination of metal ions and their adsorbents [29]. In addition, polysaccharides can associate, by physical and chemical interactions, with a wide range of other polymers [30]. Some bioactive polysaccharides have been broadly used in various applications [31-33]. Recently, several works have focused on studying the absorption capacity of metal ions with polysaccharides such as pectins, chitosan, chitin and its derivatives, modified cellulose, and alginate. These works have shown an excellent capacity for fixing metal ions on these different biopolymers [29, 34-37].

In this work, we employed a sensitive surface to detect metal ions made of hemicellulose fibers from polysaccharides isolated from rackets (cladodes) of the prickly pear "*Oputinia ficus-indica*" from north-eastern Algeria. We demonstrate an interest in such biopolymers as sensors regarding their nanostructured morphology, their resistance in an acidic medium and their multiple functional groups, which allow the capturing of metal ions. Thus, a sensitive part of the biopolymer leads to heavy metal detection in an aqueous medium. The hemicellulose fibers are integrated into an agarose gel, another polysaccharide, to form the sensor to improve its stability and chemical characteristics. Regarding the physicochemical properties of the sensor, chemical properties and detection mechanisms are studied and examined by FT-IR, AFM, SEMs, EDX, and electrochemistry. We tested the detection of heavy metals  $Pb^{2+}$  in PBS solution and real samples in water effluent with various contamination levels to highlight their behavior for various water environments.

## **1. Materials and methods**

### **1.1 Reagents and solutions**

All reagents are analytical grade and were used without further purification.

Ethanol, acetone, sulfuric acid, sodium hydroxide, and acetic acid with 99 % purity were purchased from Sigma-Aldrich. Agarose (AG) with 99 % purity was purchased from Fisher Scientific GSA. The phosphate buffer solution (PBS) was prepared from monopotassium and dipotassium phosphate salts, sodium and potassium chlorides and adjusted to pH 4. Standard solution of lead nitrate ( $\text{Pb}(\text{NO}_3)_2$ , 99%) was purchased from Fluka, nickel nitrate  $\text{Ni}(\text{NO}_3)_2$  99%, mercury nitrate  $\text{Hg}(\text{NO}_3)_2$  99%, and copper nitrate  $\text{Cu}(\text{NO}_3)_2$  99% were obtained from Sigma-Aldrich.

## **2.2 Hemicellulose Extraction**

The hemicellulose (HEM) is extracted from prickly pear cladodes according to the Habibi et al and Yang et al protocol [38, 39] with some modifications.

The dried rackets are pre-treated successively with petroleum ether and ethanol for 24 hours at room temperature with gentle stirring and then filtered in order to remove the compounds that are soluble in ethanol and petroleum ether, such as polyphenols, simple oses and amino acids. The pre-treated residue is dried in the dark with open air ventilation at room temperature for 24 hours. Hemicellulose is extracted using a 0.5 M sodium hydroxide solution NaOH at 80 ° C for two cycles of 2 hours. The combined alkaline extracts are neutralized with 20% (v / v) acetic acid and kept for 24 hours at 4 ° C. The soluble alkali fraction obtained is washed twice with ethanol and then with acetone and dried at 50 ° C. The procedure of extraction is described in SI.1, see Figure I.

## **2.3 Sensor formation modified electrode Au/Agarose-Hemicellulose (Au/AG-HEM)**

The gold electrodes (Au) used for characterisation and sensor formation were supplied by the French network RENATECH (LAAS, CNRS TOULOUSE). The electrode surface was cleaned in acetone, incubated in a solution of piranha (70%  $\text{H}_2\text{SO}_4$  / 30%  $\text{H}_2\text{O}_2$ ) (V / V) and washed with distilled water before being dried under nitrogen flux. The sensor used in electrochemical detection is a gold electrode (300 nm of gold layer/30 nm of titanium layer/300 nm  $\text{SiO}_2$ /p-type Si/300 nm  $\text{SiO}_2$ ) with an active surface defined by an o-ring seal: 0.2  $\text{cm}^2$ . The Agarose gel (0.5%) is prepared in PBS buffer (50mM), and then heated to 100 °C. The mixture of agarose/ hemicellulose (AG/HEM) (1%) was prepared by adding 10 mg of the hemicellulose powder to 1 mL of agarose solution to allow the inclusion of the hemicellulose in the gel. 15  $\mu\text{L}$  of the mixture are deposited on the electrode surface by spin-coating and dried for 24 hours at 4 °C.

## **1.4. Methods**

FT-IR spectra (4000 to 600  $\text{cm}^{-1}$ ) were recorded on a Bruker VERTEX spectrometer with a nominal resolution of 4  $\text{cm}^{-1}$  using Attenuated Total Reflection (ATR) with a crystal of Germanium and MCT detector.

Atomic force microscopy (AFM) images were obtained with tapping mode topography and phase imaging using an Innova AFM Bruker with NanoDrivev8.02 software. Tapping mode images were acquired using silicon tips from Nanosensors (PPP NCSTR) with a resonance frequency ranging between 76 and 263 kHz and a spring constant of 1.2-29 N/m.

Scanning electron microscope (SEM) micrographs and energy dispersive X-ray (EDX) spectra were recorded using a FEI Quanta 200 Environmental SEM.

$^1\text{H}$  and  $^{13}\text{C}$  NMR spectra were recorded on a Bruker Advance 300 MHz apparatus. The chemical shifts are given in ppm according to dimethyl sulfoxide- $d_6$  ( $d_6$ -DMSO) using tetramethylsilane as an internal reference.

The crystallinity of the extracted hemicellulose samples was studied by X-ray diffraction at room temperature using a Bruker AXS D8-Advance diffractometer (CuK $\alpha$  radiation) ( $k = 1.54056 \text{ \AA}$ ) at 45 kV and 40 mA equipped with a curved graphite monochromator in the secondary beam. Data was collected in a range of  $2\theta = 10$ -80.

Voltammetric measurements were performed with the EmStat<sup>3+</sup> potentiostat from PalmSens BV (Houten, Netherlands). Electrochemical impedance spectroscopy (EIS) was recorded with a Metrohm Autolab PGstat 12, controlled by Nova® software in solution of 5 mM  $[\text{Fe}(\text{CN})_6]^{3-/4-}$  containing 0.1 M KCl as a redox probe and at frequency range from 0.1 Hz to 100 kHz and DC potential and at the potential of of 5 mM  $[\text{Fe}(\text{CN})_6]^{3-/4-}$  containing 0.1 M KCl as a redox probe at frequency range from 0.1 Hz to 100 kHz and DC potential of 10 mV and applied potential of 0.2 V around the value of open circuit potential to have small current flowing and obtain a stability of the impedance signal in low frequency range. A cell with three conventional electrodes was used, with a reference electrode (Ag/AgCl/KCl (sat)), an auxiliary platinum electrode with an active surface of 1  $\text{cm}^2$  and a gold working electrode (active surface defined by an o-ring seal: 0.2  $\text{cm}^2$ ). The electrochemical measurements are carried out in a 5 mM PBS buffer solution at pH 4. For Square Wave Voltammetry (SWV), we optimized the starting potential, the end potential, the deposition potential, and the frequency (See SI.3). The deposition time was also optimized and was fixed to 280 s and the amplitude to 50 mV/s. The measured of current at peak potential was obtained after baseline treatment.

## 2. Results and discussion

## 2.1 Characterization of hemicellulose

The diffractogram of hemicellulose does not exhibit a horizontal basic line (see SI.1, Figure S1). This shows that the significant part of the matter is an amorphous structure, as a large diffraction peak was observed at  $2\theta = 14^\circ$ . In addition, the dehydrated hemicellulose shows a distinct crystalline peak at  $31^\circ$  [40]. The peak could be related to a cellulose during the purification process. Indeed, hemicellulose in the plant cell wall is a heterogeneous polysaccharide with a relatively low molecular weight and a fibrillar structure. Hemicellulose is tightly bound to the surface of cellulose microfibrils by a strong non-covalent hydrogen bond. Hemicelluloses have an amorphous structure, while cellulose is crystalline. In the case of alkaline extraction with NaOH, this method provides good hemicellulose quality while retaining the fibrillar structures, but a trace of cellulose may be present [41] which explains the appearance of crystalline zones in DRX analysis. In another way, less arabinose in hemicellulose's chemical structure could increase its crystallinity [42].

The  $^{13}\text{C}$  NMR spectrum (See SI.2, Figure S2) shows the presence of the 6 characteristic signals at 105.76 ppm (C-1), 71.36 ppm (C-2), 73.20 ppm (C-3), 77.82 ppm (C-4), 74.85 ppm (C-5), and 60.84 ppm (C-6). Compared to the cactus polysaccharides [43, 44], these specific  $^{13}\text{C}$  signals were attributed to resonance peaks characteristic of the (1  $\rightarrow$  4)-linked  $\beta$ -D-Xylp units. The weak signals at 97.61, 82.2, 73.2, 72.62, 71.36, and 60.84 ppm originate from C-1, C-4, C-3, C-5, C-2, and  $-\text{OCH}_3$  of 4-*O*-methyl- $\alpha$ -D-glucuronic acid (4-*O*-Me- $\alpha$ -D-GlcpA) units, respectively [45,40]. As shown in Figure 2, other less intense  $^{13}\text{C}$  signals were identified at 107.46 ppm, 81.26 ppm, 76.57 ppm, 84.07 ppm, and 61.19 ppm, corresponding respectively to the terminal C-1, C-2, C-3, C-4, and C-5 residues (T-D-Araf-(1,3) type) linked to galactose residues in the main galactan chain. In addition, less intense signals were observed at 103.74 ppm, 82.22 ppm, 78.14 ppm, and 72.82 ppm corresponding to C-1, C-3, C-4, C-2 of 3-*O*-D-Galp substituted residues, respectively [44, 45].

The chemical structure of hemicellulose was analysed by FTIR and Figure 1 shows the spectrum after purification of hemicellulose compared to agarose and AG/HEM composite. Figure 3a corresponding to FTIR spectrum shows various vibration peaks with a well broad and intense band centred at about  $3400\text{ cm}^{-1}$ , attributed to the OH hydroxyl group's elongation vibration characteristic of hemicellulose [47, 48]. Two asymmetric vibrational bands of C-H bonds of the pyranose group in hemicellulose are observed at  $2917\text{ cm}^{-1}$  and  $2850\text{ cm}^{-1}$ . The bands at  $1416\text{ cm}^{-1}$ ,  $1323\text{ cm}^{-1}$  and  $1047\text{ cm}^{-1}$  are associated with hemicellulose [49] and correspond to the presence of the  $\beta$ -glycosidic linkages between xylose units [50]. The intense band at  $1635\text{ cm}^{-1}$  corresponds more specifically to the elongation vibration of the C=O carboxyl group's function and confirms the presence of the galacturonic acid (Barka et al. 2013)[51]. The area between  $800$  and  $1200\text{ cm}^{-1}$  corresponds to the carbohydrate fingerprint region, and it helps in identifying some characteristics of polysaccharides functional groups, such as

stretching (C-O-C), bending (O-H), and distorting vibrations (CH<sub>3</sub>). Wavenumbers 1165 cm<sup>-1</sup> and 1047 cm<sup>-1</sup> show two bands attributed to arabinoxylans, indicating that the main bonds are of the α-glycosidic type. [52]. The characteristic band vibration of the product demonstrates the purity of the hemicellulose obtained, free of lignin, as the characteristic aromatic ring vibration at 1525 cm<sup>-1</sup> corresponding to lignin is not observed. [53].

## 2.2 Sensor formation and optimization

The sensor was formed by spin coating a combination of hemicellulose and agarose onto the surface of a gold electrode, resulting in a stable adsorbed film. The gold surface was selected as the electrode due to its electrical properties and high adsorption ability. Agarose forms a stable membrane thanks to its gelling properties. The amount of hemicellulose in agarose gel was optimized to obtain the high loading of metal ions into the biopolymer composite with a good stability (See SI.3.1).

### 2.2.1 Structural and morphological characterisations of the sensor

To confirm the association of agarose with hemicellulose, the FTIR spectrum of the composite was obtained and compared to the agarose spectrum (Figure 1A). The peaks associated with agarose (Figure 1A, curve b) are obtained at 3488 cm<sup>-1</sup> (stretching O-H), 2946 cm<sup>-1</sup> (stretching C-H), and 1064 cm<sup>-1</sup> (stretching vibration of the glycosidic bond C-O-C). The characteristic peak at 1631 cm<sup>-1</sup> is related to the carboxyl groups in the agarose gel as well as the water-related stretching vibration bond H-O-H, around 983 cm<sup>-1</sup> and 860 cm<sup>-1</sup>, a characteristic band of (3,6-galactose-anhydro) is observed [46]. Following sensor and nanogel hemicellulose/agarose formation, both the characteristic peak vibrations of hemicellulose and agarose appeared in the FTIR spectrum, confirming the encapsulation of hemicellulose into agarose gel. (Figure 1A, cure c).

To confirm the detection of Pb<sup>+2</sup>, FT-IR spectrum was recorded after the electrode was immersed in a solution of lead ions (Figure 1B, curve b). Some modifications in the spectrum were observed. Indeed, a slight shift of hydroxyl and carbonyl band was observed, indicating the coordination of Pb<sup>+2</sup> ions to OH and C-O groups [54].

AFM images of AG/HEM composite films and AG are shown in Figure 2. The AG film compared to a gold electrode (Figure 1A image a and b) shows a dense, smooth fibrous network structure. The introduction of hemicellulose in agarose film, leads to fibrous network structure with a regular distribution on the surface (Figure 2, image c). This is related to the distribution of HEM nanofiber in the agarose gel matrix, indicating that intermolecular binding between agarose and hemicellulose occurred during the gelation process. The morphology shows the same behaviour as reported previously in the case of cellulose nanofiber composite [41]



In the presence of  $\text{Pb}^{2+}$  ions after detection, the appearance of agglomerations corresponding to the complex HEM/  $\text{Pb}^{2+}$  is observed (Figure 2, image d), also confirmed by SEM images.

SEM Images were taken at two different magnification scales to determine the structural properties of AG, AG/HEM composite films and AG/HEM/ $\text{Pb}^{2+}$  (Figure 3). In AG film (Figure 3, image a) a smooth and uniform surface with many pores was observed on the surface of the dried gelled material. This result underlines the porous structure of agarose, facilitating the diffusion of molecules into the gel. In the case of AG/HEM SEM image (Figure 3, image b), the morphology image reveals stripes showing the fibrillary structure of hemicellulose warped with agarose gel. The result demonstrates the ability of hemicellulose to disperse and form a film with irregular shape and size, maintaining its original structure.

Figure SI2.d shows the EDX images of the AG film and AG /HEM. They show the peaks of carbon, oxygen and percent impurities of sulfur, chlorine, potassium, sodium, and phosphorus according to their binding energy. These elemental impurities are due to the extraction, and purification processes of the hemicellulose (HCl, EDTA, NaOH) and the preparation of the AG and AG /HEM film like as Phosphate-Buffered Saline PBS buffer  $\text{KH}_2\text{PO}_4$ , NaCl, KCl,  $\text{Na}_2\text{HPO}_4$ . Any lead signal is observed in these samples before lead interaction.

After the loading of  $\text{Pb}^{2+}$ , SEM images showed a change in the structural conformation (Figure 3, image c), the restructuring of the surface with the appearance of spherical agglomerations, and the flower-like structures. The energy dispersion X-ray microanalysis (EDX) was performed to underline the presence of lead in the surface (Figure 3, curve d). In the agglomerate structure, the spectrum shows the presence of peaks related to the elements Au, C, O, N, and  $\text{Pb}^{+2}$  related to agarose, hemicellulose and loaded lead. This could be attributed to the formation of the complex AG/HEM and  $\text{Pb}^{2+}$  after loading.

In order to obtain high performance of the sensor AG-HEM for the detection of lead ions, we carried out a series of optimization experiments regarding the sensor formation and the various parameters influencing the loading of lead and their effects in electrochemical detection. Thus, we studied the AG-HEM composition to determine the optimum ratio between AG and HEM to obtain high loading properties for metal ions loading and a stable nanogel membrane (See SI3.1, Figure S3A). Thus we demonstrate that the ratio of AG-HEM of 1% (w:w) lead to the best properties in term of detection and stability of the layer. The effect of pH on the absorption ability of the sensor was also studied in the range of 2 to 7, because the ionic properties of the material should affect the loading ability (See SI.3.3, Figure S3B). Thus pH 4 is demonstrated as the best medium to obtain high metal ion loading in the biopolymer. Additionally, the optimisation of various electrochemical parameters in SWV measurement was studied, such as deposition potential and deposition time (See SI.3.3, Figure S3C-

D), to obtain high signal intensity after the absorbed leads in the biopolymer. These optimisations are discussed and detailed are proved in SI 3.

### 2.2.3 Electrochemical characterization of the sensor (Au/AG-HEM)

Cyclic voltammetry was used to study the electrochemical process behaviour of the sensor and its electron transfer ability. Thus, the electrochemical process measured by cyclic voltammetry of the redox probe  $[\text{Fe}(\text{CN})_6]^{3-/4-}$  was studied with the various modified AG and AG/HEM electrodes and compared to the bare gold electrode in 5 mM  $[\text{Fe}(\text{CN})_6]^{3-/4-}$  with scan rate  $80 \text{ mV}\cdot\text{S}^{-1}$  (Figure 4 A). The CVs show a quasi-reversible redox signal, with a redox potential at 0.35 V and a current ratio of about 1.16 between the anodic and cathodic peaks with the gold electrode. The functionalization of the gold electrode through the AG or AG/HEM membranes shows a decrease in redox current and a shift of redox potential to 0.3 V and 0.29 V for the AG and AG-HEM composite, respectively. The result indicates that the interfacial reaction with  $[\text{Fe}(\text{CN})_6]^{3-/4-}$  was modified with functionalization [55]. The small decrease of current after the AG/HEM immobilization underlines the high porosity of the AG/HEM layers, allowing the diffusion of the redox marker on the membrane. Kinetic characterisations were performed by measuring cyclic voltammetry at various scan rates ranging from 20 to  $160 \text{ mV s}^{-1}$  with the modified Au/AG/HEM electrode in PBS, containing the redox couple  $\text{Fe}(\text{CN})_6^{3-}/\text{Fe}(\text{CN})_6^{4-}$  (Figure 4B). The CV obtained with Au/AG-HEM shows an increase of peaks current with the increase of the scan rate. The anodic and cathodic peaks currents ( $I_{pa}$  and  $I_{pc}$ ) were plotted versus the square root of the scan rate (Figure 4 C) to highlight the diffusion process on the biolayer. Both  $I_{pa}$  and  $I_{pc}$  vary linearly with the square root of the scan rate for the scan rate measured between 20 to  $160 \text{ mV s}^{-1}$ , indicating that the redox process occurring at the modified electrode was a diffusion-controlled process. Such results demonstrate that the modification of the surface by the biopolymers didn't affect the diffusion of the redox marker due to the high porosity of the biolayer. Electrochemical impedance spectroscopy EIS was also performed to investigate the electrical properties of the layer. EIS measurements were performed in presence of the various modified surfaces AG and AG-HEM and compared to a non-modified gold surface (Figure 5).

A Nyquist plot showed an enlargement of the semicircle diameter as a function of the modified surface with an increase of semicircle diameter (Figure 5 A). This parameter is related to the charge transfer resistance ( $R_{ct}$ ). The linear part in the lower frequency range represents the Warburg impedance related to the diffusion process and it shows a diffusion of the redox marker, demonstrating the porosity of the biopolymers[56].

The EIS data were fitted with Randles equivalent circuit, and data are presented in (Table1). An increase in the charge transfer resistance ( $R_{ct}$ ) after immobilisation of the AG, AG-HEM film on the gold electrode surface was calculated compared to the non-modified surface (Table 1). The  $R_{ct}$  of the

modified AG and AG-HEM electrodes are 482  $\Omega$  and 670  $\Omega$  compared to the gold surface obtained at 231  $\Omega$ . The result indicates that electron transfer was slowed down by the addition of AG and hemicellulose [57]. The presence of negatively charged functional groups, such as carboxylic acid and hydroxyl groups prevent the diffusion of negatively charged the redox marker. This result is also consistent with the CV measurement shown in Figure 4A.

After the detection of lead ions, ESI was measured, and it shows that the diameter of the semicircle of the Nyquist plot decreased (Figure 5B). The Rct value measured after fitting with the same circuit decreases to 594  $\Omega$  (Table 1). This could be explained by the presence of the positively charged lead ions  $Pb^{2+}$  in the polymeric membrane favouring the charge transfer of the redox marker.

### 2.3. Electrochemical lead detection

In order to test the sensitivity of the sensor Au/AG-HEM, two electrochemical methods were used, cyclic voltammetry (CV) and square wave voltammetry (SWV). Different concentrations of  $Pb^{2+}$  ions ranging from 10 fM to 1  $\mu$ M were analysed. These two methods follow the redox behaviour obtained on the electrode surface highlighting the amount of lead ions entrapped into the membranes.

The cyclic voltammetry measurements show the redox signal of lead ions and current oxidation and reduction variation upon increasing concentration (See SI 4 Figure S4). The voltammograms show two redox peaks with a cathodic peak at 0.2 V, attributed to the reduction of the metallic cation accumulated on the surface of the modified Au/AG/HEM electrode, and an anodic peak at 0.3 V, attributed to the oxidation or re-dissolution of the metal. It can be observed from the recorded CV measurements that the peak current intensities increase with the concentration of a lead ion in solution, which is attributed to the amount of absorbed  $Pb^{2+}$  on the membrane on the modified electrode. Compared to other sensors, where lead oxidation is observed at negative potentials between -0.4 and -0.6 V vs. Ag/AgCl, the observed redox potential of the oxidation and reduction peaks shows a significant shift to a higher potential [60]. This potential shift is probably due to the significant retention effect of the metal in the membrane.

The mechanism of association of metal ions and their detection could be explained as follow. When the electrode is in contact with metal ions, the adsorption process by complexation is likely to take place, either with the formation of a chemical bond (ionic) established via a partition based on the electrons pooling present in the outermost orbitals or of an electrostatic bond between the surface and the metal cation. The ability of the functional groups  $-COO^-$  and  $-OH$  to retain metal ions is significantly affected by the pH of the solution. The dissociation constant (pKa) of many functional groups, including carboxylic groups, varies between 3.5 and 5.5, which means that most of these groups will be deprotonated in this pH range and an increased number of negatively charged sites will be available for the interaction of metal ions and their absorption [61]. The study of the pH effect

confirms this where 4 is found to be the optimum pH for lead absorption (See SI3). This absorption phenomenon can occur in two steps: In the first step, the heavy metal ions migrate by diffusion from the aqueous solution to the adsorbent surface, which contains an opposite charge. Then, once the heavy metal ions have passed through the boundary layer, they attach to the adsorbent surface. This type of absorption is often achieved by Van Der Waals forces, dipolar interactions, hydrogen bonds, or ion exchange which, consists of the diffusion of the metal ions towards the ligand [62, 63]. The tendency to ligation depends on the charge and the relative size of the metal elements exchanged [64].

### 2.3.1 Sensor performance

To evaluate the sensitivity of the biopolymer to lead detection, SWV was measured for different lead concentrations from 10 fM to 1  $\mu$ M in the same conditions as for CV. The oxidation peak of lead ions is obtained at 0.3 V and the peak current intensity increases with the concentration of  $Pb^{2+}$  in the solution confirming the results obtained with CV. The signal increase is attributed to the association of  $Pb^{2+}$  cations in the membrane and highlights the high absorption capacity of the membrane.

The calibration curve plots the peak current after baseline treatment variation versus the log concentration scale. It shows a variation with a wide range from 10 fM to 1  $\mu$ M with a regression coefficient of 0.992 (Figure 6 B) and a sensitivity of 10  $\mu$ A/decade. The detection limit was determined according to a method based on a signal-to-noise ratio of 3 and the standard deviation of the blank test obtained with five independent sensors and divided by the slope of the calibration curve obtained with linear part of the curve following the formula ( $LOD = 3 \times SD \text{ blank} / \text{Slope}$ ). A value of 1.3 fM is calculated. It could be seen from the SWV curve for the first concentration that the signal obtained with the concentration of 10fM gives an intensity of peak current around 23 $\mu$ A, which is higher compared to blank test (0.5 $\mu$ A). Test the detection capacity with the agarose hydrogel for  $Pb^{2+}$  and the interest of adding the biopolymer hemicellulose, the same experiments were performed with the same concentration range using SWV for electrochemical measurement. Agarose gel also allows the loading of lead in the biopolymer. However, the detection limit is measured to be 1 pM (See SI, Figure S5). The result demonstrates that the association of the two biopolymers and the richness of hemicellulose in functional groups improves the ability to detect lead with higher sensitivity and lower LOD.

For the selectivity test, the modified Au/AG-HEM electrode was used to measure the presence of  $Pb^{2+}$  in the presence of other metal ions such as  $Ni^{2+}$ ,  $Cu^{2+}$ , and  $Hg^{2+}$  using SWV. The measurement was performed with 1 $\mu$ M of  $Pb^{2+}$  and 10 $\mu$ M of the other metal ions. The current obtained at the potential of lead oxidation was plotted (Figure 6C). The result shows that the other metal ions slightly affect the sensor's response, with 8% of current variation occurring when other metal ions are

introduced at higher concentrations. This result demonstrates the selectivity of the biopolymers to lead ions compared to other metal ions. This result could be explained by the coordination sphere in the biopolymers favouring lead interaction over that of other metal ions.

Compared to the other sensors described in the literature (Table 2), this sensor formed with biopolymers AG-HEM demonstrates a high ability to detect lead ions with high sensitivity, a wide range and a detection limit of 1.3 fM. This behaviour is explained by the affinity of the  $\text{Pb}^{2+}$  cations to the hydroxyl and carboxyl groups in hemicellulose, which leads to metal cation binding, as demonstrated by the FTIR measurement.

### **2.3.2 Reproducibility, repeatability and stability**

We evaluate the performance of the developed sensor such as repeatability, reproducibility, and stability by repetitive measurements. The repeatability was carried by measuring six consecutive measurements with the same sensor with 0.1 nM of lead solution. The current variation was recorded and a relative standard deviation of 4.4% was measured.

The reproducibility was studied with five fresh sensors by measuring the same range of concentration of lead from 1 fM to 1  $\mu\text{M}$ . The standard deviation was measured at 6.5%.

We can assume that the sensor exhibits a good reproducibility and repeatability.

The stability was studied by measuring the sensor's response during 20 days in the same conditions (see SI5, Figure S6). The sensor retains 90% of the response after 20 days of storage at 4°C.

### **2.4 Application on a real sample**

To demonstrate the ability of the sensor to detect lead ions in real samples, we performed a serial measurement directly in samples from various rivers and sea which should have different level of contamination without any treatment, filtration or addition of other compounds. The measurement was performed using the standard addition method. Firstly, the blank measurement was performed and then various lead concentrations were added and measured. The sample for the river with high contamination was a river in Paris France where high concentration of lead ions could be detected. As is well known, the Notre-Dame de Paris Cathedral's roof and spire, which caught fire and fell in April 2019, was covered in 460 t of lead (Pb). Government reports documented a Pb deposition immediately downwind of the cathedral and a twenty-fold increase in Pb concentrations in the air, water and soil at a radius of 50 km after this accident [67, 68]. The experiment was performed using a standard addition method with three different measurements. The modified electrode Au/AG-HEM sensor was dipped directly in the water sample of river water, and the sensor's response was measured directly. Then, various concentrations of lead ions were added to the river water and the

electrochemical response was recorded. SWV measurements (Figure 7A) show the redox peak at 0.3V corresponding to the signal of lead ions absorbed on the biopolymers. The signal obtained after the added concentration is lower than that obtained in PBS solution due to the absence of the buffer solution and electrolyte. The peak current value increases with the increasing of concentration of added lead in the river water. The current value indicates the level of  $\text{Pb}^{2+}$  in the solution. The calibration curve presenting the variation of current with the concentration of the added  $\text{Pb}^{2+}$  is then plotted (Figure 7B). Linear variation is measured and the intercept of the curve allows determining the level of  $\text{Pb}^{2+}$  in river water. Thus, concentration of  $\text{Pb}^{2+}$  measured is  $0.43 \mu\text{M}$  ( $89 \mu\text{g/L}$ ), with an RSD of 8.54%. The value obtained is significant and is in the correlation of reported data concerning the seine river contamination with leads where  $27\text{-}40\mu\text{g/L}$  is measured for leads before the fire[67, 68].

To evaluate the effectiveness of using Au/AG-HEM for the determination of  $\text{Pb}^{2+}$ , various real water samples were measured. This study include water from river of Paris countryside (Yvette) which known to have low contaminated of lead and three different water samples from JIJEL a mountain region of Algeria known to have low lead concentration. From the mountain region, three different water samples from various environment seawater, spring water and tap water were measured. The lead determination in these sample were performed with the same method using the standard addition method (see SI, Figure S7). The  $\text{Pb}^{2+}$  concentration in water sample was measured at  $12\text{nM}$  ( $2.4\mu\text{g/L}$ ) in river of countryside of Paris and at  $0.12\text{nM}$  ( $0.024\mu\text{g/L}$ ) for sea water,  $0.21\text{nM}$  ( $0.043\mu\text{g/L}$ ) for spring water and  $2.7\text{nM}$  ( $0.55\mu\text{g/L}$ ) for tap water in mountain region.

These results demonstrate the ability of this sensor to measure directly both high concentration and ultra-traces of the lead in water without any sample treatment in various environment using standard addition method.

## Conclusion

We demonstrated that the sensor formed with hemicellulose extracted from the *Opuntia Ficus Indica* cactus associated with the agarose meets current requirements detecting lead ions traces in water. The sensor combines agarose's conductive behaviour with the loading properties of hemicellulose. We highlight that the new composite provides a synergistic effect for the detection of the lead ions with high sensitivity where a detection limit of  $1.3 \text{ fM}$  was measured. The sensor exhibits good performance regarding selectivity, repeatability and reproducibility for  $\text{Pb}^{2+}$  ions. Selectivity was determined regarding other divalent metal ions such as  $\text{Ni}^{2+}$ ,  $\text{Hg}^{2+}$ , and  $\text{Cu}^{2+}$  and showed the small effect of these metal ions on the sensor's selectivity. Finally, the sensor was applied to determine  $\text{Pb}^{2+}$

directly in real samples of river water and sea without any previous treatment. The sensor demonstrates their ability for direct measurement. The results showed that this system offers great promise for lead detection and could be applied for monitoring lead contamination directly in various water environment samples.

## Acknowledgements

Authors thank the General Directorate of Scientific Research and Technological Development (DGRSDT) for their financial support and MEAE MESRI for funding PHC project N 39382RE.

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