



Characterization and Application of Carbon Paste Electrode Modified by *Moringa Oleifera* Seed Powder to the Electrochemical Detection of Mercury

**Kouamé Jean Marc N' Guessan ^a, Seiny Roger N'Dri ^{b*},
Yapi Hermann Arstide Yapo ^a, Légré Paul Gauly ^a,
Ali Sanou ^{b,c,d}, Segnihintenin Bakary Soro ^{b,c},
Mariame Coulibaly ^b, Drissa Bamba ^b
and N' Guessan Alfred Yao ^b**

^a Jean Lorougnon Guédé University of Daloa, UFR Environment, Science Laboratory and Environmental Technologies (LSTE), BP 150 Daloa, Ivory Coast.

^b School Normal Superior of Abidjan, Department of Science and Technology, Laboratory of Fundamental and Applied Physical Sciences (LSPFA), Group of Research in Electrochemistry (GRE), 08 BP 10 Abidjan 08 Abidjan, Ivory Coast.

^c Félix Houphouët-Boigny University, UFR-SSMT, Constitution and Reaction Laboratory of Matter (LCRM), 22 BP 582 Abidjan, Ivory Coast.

^d National Polytechnic Institute Félix Houphouët-Boigny, Process Laboratory Industrial Syntheses of the Environment and New Energies, BP 1313 Yamoussoukro, Ivory Coast.

Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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*Corresponding author: E-mail: ndri.roger@ensabj.ci, rogesny@yahoo.fr;

ABSTRACT

Heavy metal pollution of surface waters from anthropogenic activities is constantly increasing. Monitoring techniques must therefore be more precise and more sensitive. One of the effective methods available is the development of sensitive electrochemical sensors, dedicated to the analysis of pollutants in aqueous solution. The carbon paste electrode (CPE) in this study was modified with *Moringa Oleifera* seed powder, a flocculant-adsorbent used to treat water. This chemically modified electrode presented improved electrochemical reactivity in cyclic voltammetry towards the redox marker Ferri / Ferrocyanide compared to the bare carbon paste electrode. The 10 % modifier presented the most interest during the analysis. The Modified electrode was used to detect mercury (II) in anodic stripping voltammetry. The detection current of mercury (II) is seven times that of carbon paste electrode (CPE). The calibration curve is linear in the range from 0.002 $\mu\text{mol/L}$ to 0.009 $\mu\text{mol/L}$ (0.4 $\mu\text{g/L}$ to 1.8 $\mu\text{g/L}$) with a detection limit of 0.4 $\eta\text{mol/L}$ (i.e.0.08 $\mu\text{g/L}$). This new technique can be used for routine monitoring of real aqueous matrices.

Keywords: *Electrochemical sensor; Complexation; modified electrode; Mercury (II); cyclic voltammetry; anodic stripping voltammetry.*

1. INTRODUCTION

The development of artisanal gold panning over the decades in our countries has contributed to the increase in the presence of mercury in water and food [1,2]. Mercury is a chemical element whose intoxication attacks the nervous system [3] and the reproductive system [4]. Apart from its supposed use for the treatment of syphilis in the 15th century [5], caries [6] and in certain measuring instruments [7], no other use is known for it. Mercury is not biodegradable and has no known biological function [8,9]. The lethal dose is between 150 and 300 mg [10]. It therefore becomes a pollutant that must be eradicated. Its eradication in the environment requires the use of sensitive and selective materials against mercury. Initially, these materials will have to react in real matrices to the presence of mercury. One of the matrices most polluted by mercury in gold panning environments is water [1,3]. It is therefore necessary to ensure regular monitoring of this matrix. Classical methods such as Atomic Absorption Spectroscopy (AAS) [11,12], Atomic Emission Spectrometry (AES) [13] and Mass Spectrometry (MS) are expensive for most control structures [14]. An alternative has become necessary in recent decades. These are electrochemical sensors. These electrochemical sensors are very accurate and can measure heavy metal levels down to parts per million (ppm) and parts per billion (ppb) levels. This makes it an essential sensing technology for monitoring many real samples [15-17]. Once the community is alerted to the state of pollution of the matrices, it must be treated to make it suitable for use. Its treatment requires the use of

new materials that are non-toxic for the environment and for humans. In addition to this aspect, the material must be biodegradable. One of the materials used in recent times to treat water is *Moringa Oleifera* seed powder extract [18]. This material is a biodegradable plant-based flocculant-adsorbent which helps depollute water by eliminating toxic elements [18] and pathogens [19]. It also improves water turbidity without variation in pH and electrical conductivity. It also helps to enrich the treated environment with mineral salts and trace nutrients [18]. This material, in addition to participating in the treatment of residual water, can according to Zaroual et al [18] be used to eliminate heavy metals thanks to the complexation reactions of certain ions by the proteins of the *Moringa extract*. This is the reason for using seed powder as the carbon paste electrode modification material in this study. The aim of this work is to develop an ultra-sensitive electrochemical sensor for mercury using the carbon paste electrode modified with *Moringa Oleifera* seed powder extract and later applied it to wastewater discharges mining.

2. MATERIALS AND METHODS

2.1 Reagents and Chemicals Used

The chemicals used are pure analytical grade. The graphite powder comes from the company Sigma-Aldrich, the perchloric acid (HClO_4 at 70%), the sodium hydroxide (NaOH) were supplied by Prolabo. Ferri/ferrocyanide (potassium hexacyanoferrate (II) trihydrate $\text{K}_4[\text{Fe}(\text{CN})_6]$) is from Sharlau. The Dp-Pharma

company delivered the paraffin oil. The solutions are prepared with distilled water at room temperature $25^{\circ}\text{C} \pm 1^{\circ}\text{C}$. The pH of the solutions is measured with a Hanna pH meter. The Ferri / Ferrocyanoide solution has a pH value = 7 and the base solution (NaOH) pH = 13. The perchloric acid supporting electrolyte solutions have a pH = 2 for the preconcentration phase and pH = 1 for the detection phase.

2.2 Moringa Seed Powder

Moringa Oleifera serving as modifier was prepared according to the following procedure: *Moringa Oleifera* seeds were first harvested from a plant present on the site of the Ecole Normale Supérieure (ENS) of Côte d'Ivoire, then they were shelled and dried in the sun to maintain a constant weight. The sun-dried seeds were ground into powder using a blender. The powder obtained is sieved with a 500 μm sieve in order to have finer particles to facilitate mixing with the carbon powder.

2.3 Measuring Equipment and Devices

In this study, all electrochemical characterizations were carried out using a potentiostat PalmSens from PalmSens BV. The cell is connected to three electrodes: a working electrode (W), a reference electrode (R), Ag/AgCl / KCl sat and a platinum counter electrode (CE). Everything is computer controlled using the PsTrace software. The polarization curves are reproduced using Origin Pro 8 and Excel software installed on a computer on which the results obtained are recorded.

2.4 Development of the Carbon Paste Electrode

The carbon paste electrode was prepared according to the following procedure: 0.7 g of graphite powder was mixed with 0.3 mL of paraffin oil used as a binder. The mixture is ground in an agate mortar until the paste is homogeneous. Then, part of the paste obtained is compacted under mechanical control in a glass cylinder with an active surface area $\pi r^2 = 0.1256 \text{ cm}^2$. A copper wire inserted into the carbon paste ensures electrical contact.

2.5 Moringa Seed Powder

The carbon paste electrode modified by *Moringa* (CPEM-MO) is prepared by carefully mixing in

the agate mortar 0.6 g of graphite powder, 0.1 g of previously prepared *Moringa* seed powder and 0.3 mL of paraffin oil until obtaining a homogeneous paste. The resulting paste is compacted in the cylindrical body of the working electrode.

2.6 Analysis Procedure

The procedure used to analyze mercury ions is anodic stripping voltammetry. It is done in two stages. The working electrode is immersed in a solution containing the ion to be analyzed for 15 minutes with magnetic stirring at 1500 rpm: this is the preconcentration step. After this step, the electrode is rinsed with distilled water and immersed in the perchloric acid support electrolyte not containing the pollutant. The measurements are carried out with differential pulse voltammetry: this is the detection step.

3. RESULTS AND DISCUSSION

3.1. Influence of the Quantity of *Moringa* Seed Powder on the Redox Reaction of the Ferri/Ferrocyanoide Couple

The materials used in this study to make the working electrodes are carbon graphite (45 μm) and shelled *Moringa Oleifera* seed powder. Graphite carbon was used to make the bare carbon electrode. *Moringa* seed powder was used to modify the bare carbon paste electrode. The physical characterization of carbon paste is noted in numerous works [8,9]. The hulled *Moringa* seed powder used in this study was characterized by Téhé et al, [20]. The physical characterization carried out by this team with IRTF, DRX, SEM techniques of the shelled seed powder showed a high structural heterogeneity, an amorphous and porous character of the matrix. This structure would facilitate ion adsorption processes. *Moringa* is also said to be full of numerous minerals, the main ones being sodium (Na), calcium (Ca), magnesium (Mg), etc. [20]. The study of the physicochemical properties of hulled seed powders revealed that these powders constitute a good source of proteins [20]. These proteins are made up of basic polypeptides capable of neutralizing colloids in turbid water [20].

The influence of the percentage of modifier incorporated in the carbon paste on the response of the Ferri/Ferrocyanoide couple is presented in the voltammograms in Fig. 1. Current peaks are distinctly separated.

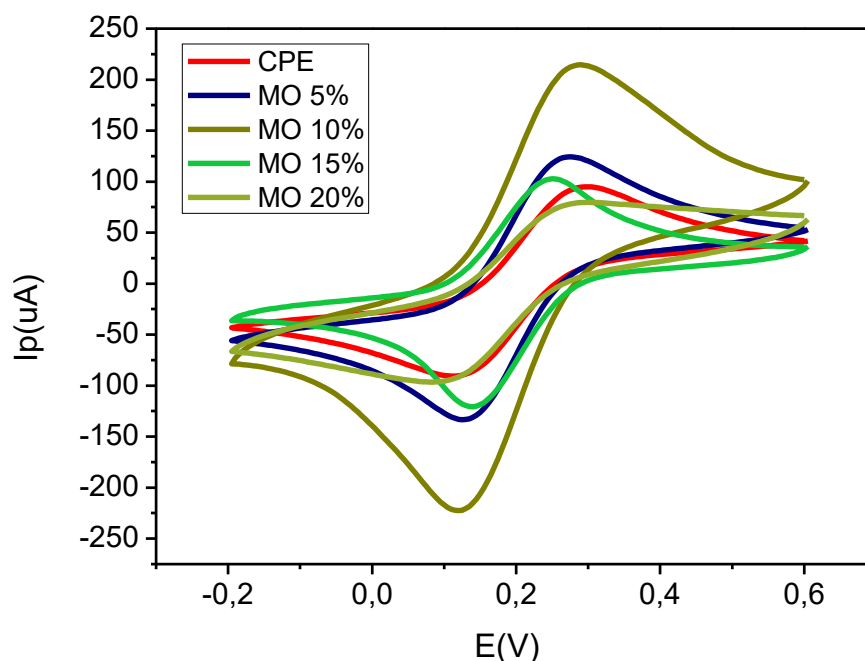


Fig. 1. Cyclic voltammograms of the response of 5.10^{-3} M of the Ferri/Ferrocyanide couple as a function of the percentage of modifier (5 %, 10 %, 15 %, 20 %) incorporated in the carbon paste electrode in a KCl electrolyte solution of concentration 0.1 M

The current peaks of each voltammogram were used to plot the graphs in Fig. 2.

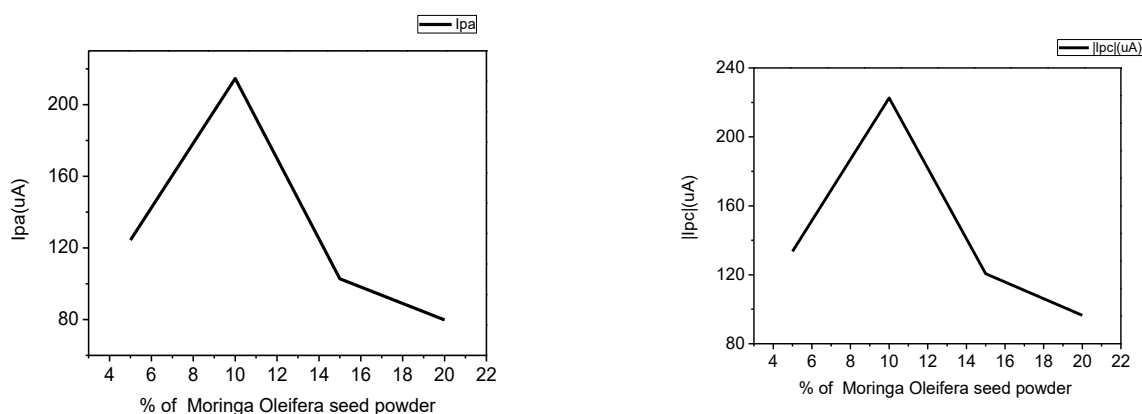


Fig. 2. Evolution of the anodic (I_{pa}) and cathodic (I_{pc}) peak currents as a function of the percentage of *Moringa* inserted in the carbon paste in 5.10^{-3} M of Ferri / Ferrocyanide in a KCl electrolyte solution of concentration 0.1 M

The curves have three appearances:

- A sudden increase in peak current between 5 % and 10 %;
- A sudden drop in peak current between 10 and 15 %;
- and a slow decrease in peak current between 15 % and 20 %.

The two curves present a maximum which is located at 10 % modification. The anodic peak current has a maximum value equal to 214.56 μ A. It is higher than other peak currents generated by 5 %, 15 % and 20 % modification. The ratios between the anodic peak currents at 10% respectively 5 %, 15 % and 20 % are 1.7; 2 and 2.6. These ratios are greater than 1. At the

cathodic peak, the evolution of the current seems almost similar. The evaluation of the current ratios gives the coefficients 1.6; 1.8 and 2.3 for respectively 5 %; 15 % and 20 %. These ratios are greater than unity. The coefficients of the current ratios evolve in the same order, this suggests that the influence of the modifier is on the overall intensity of the reaction. The drop in current beyond 10 % modification could be explained by the existence of phenomena which isolate part of the active zone of the modified electrode. This phenomenon would be linked to the lipids contained in the seed powder, the quantity of which increases with the increase in the modifier. The improvement of the overall current to 10 % seems to indicate that faradic processes are predominant compared to capacitive processes. The carbon paste electrode modified by 10 % *Moringa* seed powder which has the best electrical properties is used subsequently. The studies that will be carried out are on the analysis of the reaction mechanism in cyclic voltammetry and on the detection of Mercury (II) in anodic redissolution voltammetry.

3.2 Study of the Reaction Mechanism and Transport Phenomena of the 10% Modified Electrode

The comparative study of the response of the Ferri/ferrocyanide couple on the electrode with bare carbon paste and with 10 % modified carbon paste is presented in Fig. 3. Both voltammograms exhibit a reaction cycle. The presence of reaction cycle suggests that the materials used are active

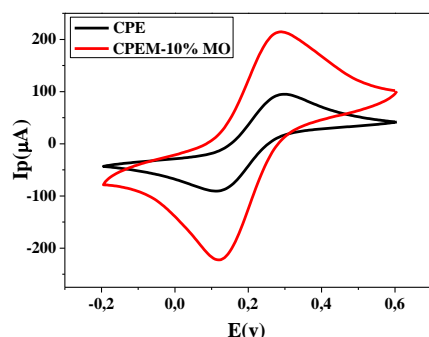


Fig. 3. Cyclic voltammograms of CPE and CPEM-MO with 10% *Moringa* seed powder on Ferri / Ferrocyanide of concentration 5.10^{-3} M in a KCl electrolyte of concentration 0.1M

In Fig. 3, the values of the anodic and cathodic current peaks of the modified electrode are maximum in absolute value than that of the bare

electrode. The modifier therefore improves the electrical properties of the bare carbon paste electrode. To evaluate the reaction mechanisms that take place during the Ferri / Ferrocyanide reactions on the two working electrodes, the quantities deduced from the voltammograms are listed in Table 1.

Table 1. Electrical parameters of the reaction of the Ferri / Ferrocyanide couple on the CPE and CPEM-MO electrode at 10 % compared to an Ag / AgCl / KCl reference electrode

electrical characteristic	ΔE_p (V)	$ I_{pa} / I_{pc} $
CPE	0.19	1.05
CPE M-MO	0.17	0.96

Analysis of the table gives the following information:

- The potential difference $\Delta E_p > 0.059$ V. This potential difference suggests that the electronic transfer kinetics is slow on the two working electrodes [8]. In addition it is faster on the modified electrode.
- The ratio between the currents $\left| \frac{I_{pa}}{I_{pc}} \right| \approx 1$ suggests that the reactions are reversible on both electrodes. These reactions are not influenced by transport phenomena at the interface [8,21].

Moringa seed powder does not significantly influence the nature of the reaction of the Ferri/Ferrocyanide couple [21-23]. Its influence is only on the anodic and cathodic current peaks. Research into the nature of the transport that occurs at the sensor interface requires the study of the peak current as a function of the square root of the scanning speeds. The result is shown in Fig. 4. The scanning speeds used are between 5 and 100 mV/s. The relationships between the peak current (I_p) and the square root of the scan speed ($v^{1/2}$).

The relationship between the peak currents and the square root of the scanning speeds is quasi-linear. This observation suggests that the reaction is quasi-reversible [9]. The mass transport process is diffusional. This process is contrary to the observation of some authors who worked with other electrode materials [8]. In the reaction process, generally, there is a stripping of an electron from the electrode material to lead to reversibility. In this study according to the work of

Zaroual et al, [18] *Moringa Oleifera* seed powder ionizes giving two protons ($2H^+$) and two electrons ($2e^-$). Therefore, during the reaction the seed powder incorporated in the paste will ionize and provide electrons in the electrochemical double layer. During the round-trip scans, the Ferri / Ferrocyanide couple always has electrons

at its disposal which will maintain the reaction. This is what seems to justify the diffusional process observed. The seed powder improves the transport of material to the surface of the working electrode. This improved electrode can therefore be used for an analytical application.

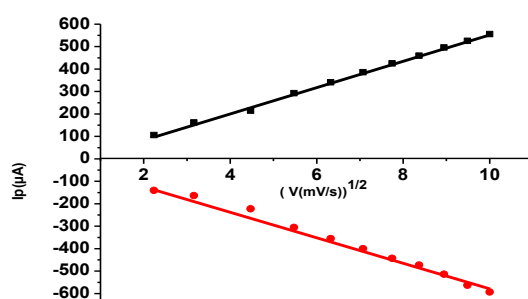


Fig. 4. Peak current I_p as a function of the root of the scanning speed ($v^{1/2}$). The cathodic peak current (red) and the anodic peak current (black)

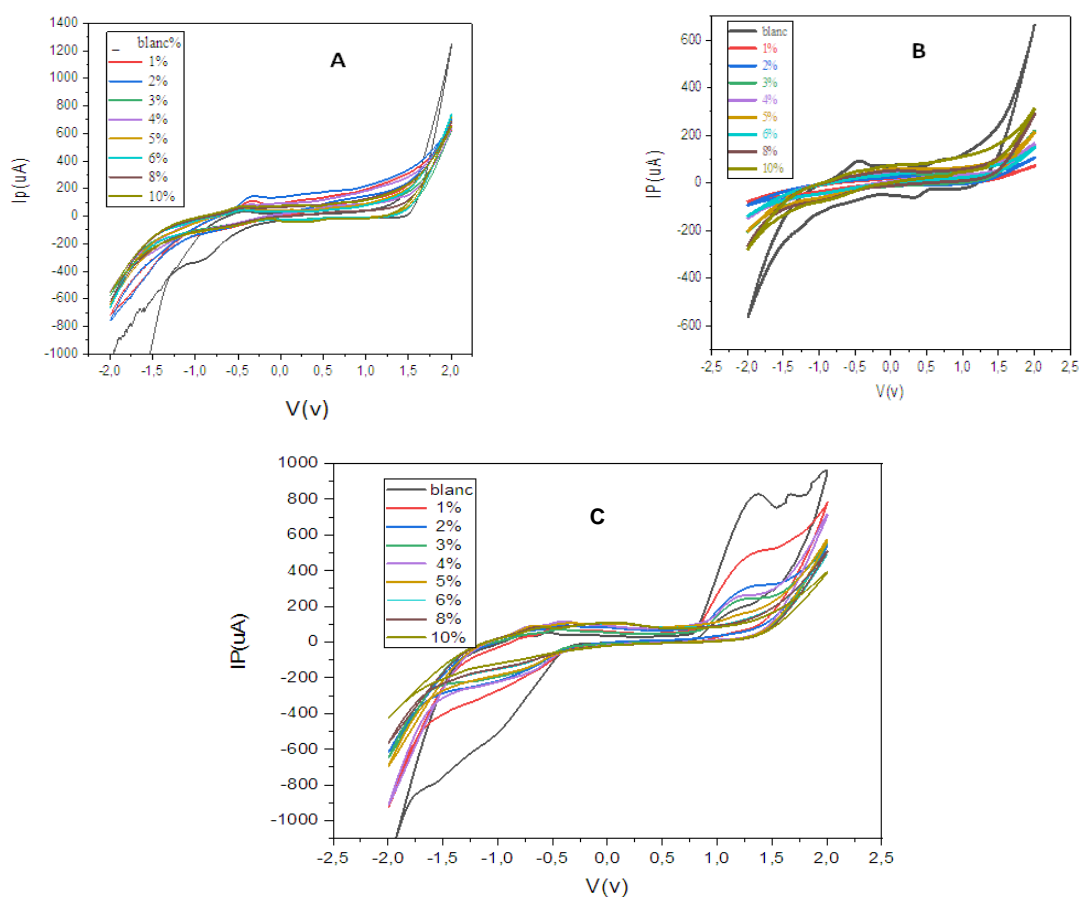


Fig. 5. Cyclic voltammograms recorded on the CPEM-MO at 25°C, A- $HClO_4$ acidic medium (0.01 M); B- neutral medium (distilled water H_2O); C- basic $NaOH$ medium (0.01 M)

3.3. Study of the Electrochemical Activity of CPEM-MO in Supporting Electrolyte

The search for a solvent that optimizes the electrochemical activity of CPEM-Mo was investigated in different supporting electrolytes. The main function of the supporting electrolyte in an electrochemical cell is to ensure the circulation of current through the transport of ions in solution [24]. It is therefore essential that the supporting electrolyte has good ionic conductivity and low viscosity to facilitate mobility [24]. If the electrolyte is aqueous, it is necessary to take into account the two populations which influence the activity of the electrode. These are water molecules that solvate free water ions and molecules. The molecules which solvate the ions are the molecules which have lost their freedom of movement and which move in solution with the solvated ion [24]. Therefore, this process results in the creation of an electrochemical double layer which acts on the sensitivity and selectivity of electrochemical sensors [21].

Fig. 5 presents the voltammograms obtained with the solvents for different percentages of seed powder incorporated into the paste. The solvents involved are perchloric acid (HClO_4), distilled water and base solution (NaOH). Figs. 5A, designate the voltammogram in perchloric acid medium, Figs. 5B, in a neutral environment and Figs. 5C in basic environment. The voltammograms show electrochemical activity in the potential window [-2 V, 2 V], with a redox peak (anodic and cathodic) for HClO_4 and H_2O and an anodic peak for the basic medium. In perchloric acid and aqueous media, the redox reaction of the incorporated seed powder appears reversible. The presence of a single peak in basic medium suggests an irreversible reaction. The analysis of voltammograms A and B shows that the anodic and cathodic peaks do not appear in the same direction from left to right. In perchloric acid, the anodic peak appears after the cathodic peak while in distilled water it is the opposite. The potential difference obtained is 0.75 V and -1 V in A and B. In absolute value the potential differences are greater than 0.057/2 V, the reaction is quasi-reversible [9]. The analysis of the current amplitudes shows high current values in the decreasing direction in acidic medium, neutral medium and basic medium. From all the investigations it appears that the perchloric acid environment seems to be favorable for the analytical application of CPEM-MO to 10 % *Moringa Oleifera* seed.

3.4 Application of CPE and CPEM-MO Electrodes to the Detection of Mercury (II) Ion

In the previous study, it was found that the perchloric acid medium is favorable for the analytical optimization of the sensor. The detection of Mercury (II) in this support electrolyte is carried out by anodic stripping voltammetry with the bare electrode (CPE) and the modified electrode (CPEM-MO). The results are shown in Fig.6.

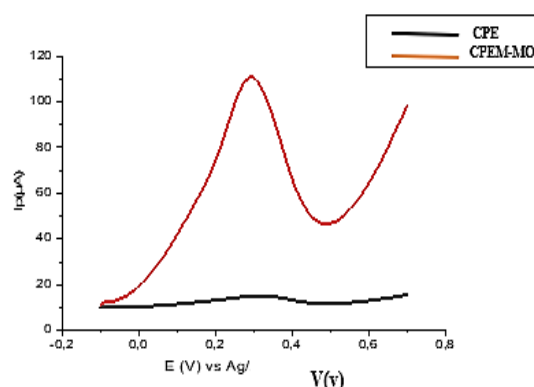


Fig. 6. Detection of mercury with a concentration of 10^{-9} M by anodic stripping voltammetry on: (in black) on the CPE, on the CPEM-MO (in red) preconcentration time 5 min, speed 30 mV/s, amplitude $E = 100$ mV and HClO_4 support electrolyte

The detection current waves obtained are presented on the same voltammogram for comparison, in black that of the CPE, in red that of the CPEM-MO. The waves appear at 0.3 V. The current amplitudes are 110 μA for the CPEM-MO and 15 μA for the CPE, respectively. The detection current of CPEM-MO is high compared to that of CPE. By comparing these two values, we see that the seed powder increases the detection power of the CPE more than seven (7) times. The increase in detection current at the carbon paste electrode modified by *Moringa Oleifera* seed powder suggests that a significant amount of mercury ions arrived at the surface of the working electrode to be detected. The modified working electrode contains mercury complexation sites on its surface. Compared to the carbon paste electrode, the active sites are provided by *Moringa Oleifera* seed powder. We can therefore suggest that the seed powder has a strong complexing power [18]. In the complexation mechanism, *Moringa* exchanges two protons for the metal ion [18]. The

incorporated seed powder improves the sensitivity of the modified electrode. Carbon paste electrode modified by Moringa seed powder (CPEM-MO) has in its active zone chemical functions complexing Mercury (II). According to Zaroual et al, [18] it is the dicarboxylic acid functions of essential oils or flavonoids, which are responsible. Zaroual et al. proposed the complexation equation for cadmium and lead [18]. That of this study is presented in Fig. 7.

3.5 Calibration Curve

The calibration curve has great importance in the analytical field. This curve shows the concentration range for which the technique is valid. It establishes a link between the detection current and the pollutant concentration. As soon as a concentration is outside the concentration window the technique is no longer valid. In addition, it allows with the detection limit to evaluate the sensitivity of the method. In Figure 8, the evolution of the peak current as a function of the mercury concentration was recorded. An increase in the mercury peak current is observed with the Mercury (II) ion concentration. To obtain a quantitative analysis method, one must be able to relate the intensity of the current to the

concentration of the target element. Thus, the calibration curve in a concentration range between 10^{-9} mol.L⁻¹ and 9.10^{-9} mol. L⁻¹ was plotted. The voltammograms and the linearity domain of the method are presented in Fig. 8.

Current peaks appear at the same potential around 0.3V. The new material developed presents stability because there is no potential shift. Figure 7b presents the linearity domain of the calibration curve. The curve is a straight line which has the equation: $I_{pa} (\mu A) = (5172.7108 \pm 115.0501) [Hg^{2+}] (\mu mol / L) + (46.8148 \pm 0.7092) (\mu A)$ ($R^2 = 0.9985$). $R^2 = 0.9985$, indicates that the method can be applied to the determination of Mercury (II) in different samples.

The analytical parameters of the developed electrode are summarized in Table 2 where the limits of detection (LOD) and quantification (LOQ) are calculated from formulas (1) and (2) [25-27].

$$LOD = 3 Sd / m \quad (1)$$

$$LOQ = 10 Sd / m \quad (2)$$

With m: the slope of the calibration line and Sd: the standard deviation.

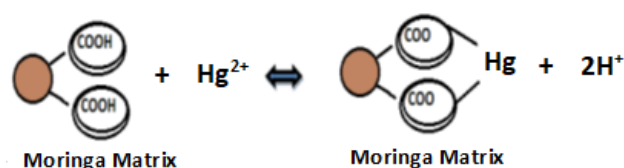


Fig. 7. Complexation reaction of Mercury ions by the Moringa matrix

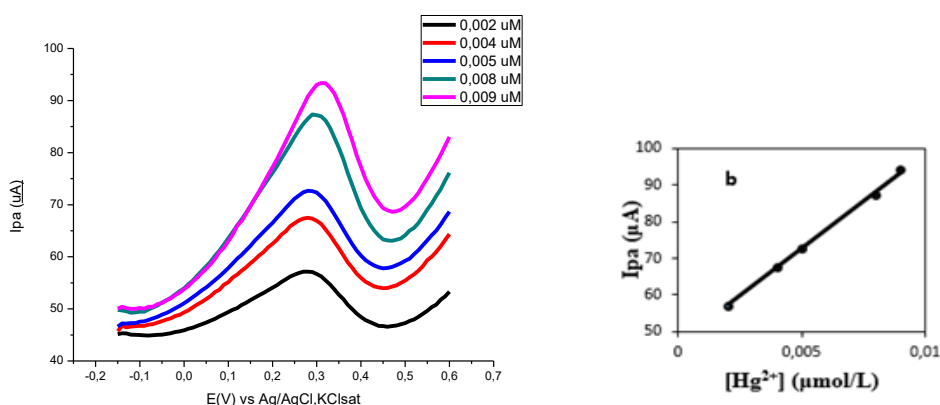


Fig. 8. a) Anodic stripping voltammetry on CPEM-MO at 10% ; preconcentration time 5 min; speed 30 mV/s, pulse amplitude $E = 100$ mV and $HClO_4$ support electrolyte at 0.1 M. b) Mercury calibration line (0.001 μ M to 0.009 μ M)

Table 2. Parameters of the linearity domain of the calibration curve

Ions	Slope ($\mu\text{A}/(\mu\text{mol/L})$)	Intercept (μA)	R^2	LOD ($\mu\text{mol/L}$)	LOQ ($\mu\text{mol/L}$)
Hg ²⁺	5172.7108	46,8148	0.9985	0.0004	0.2748

Table 3. Comparative study of some detection limit values of electrochemical mercury sensors

Working electrode	LOD (nM)	Method	References
Carbon paste electrode Modified by <i>Moringa Oleifera</i> seed powder	0.4	ASV	This work
Carbon paste electrode Modified by P-doped Silicon	5	DPV	[8]
Carbon Paste Electrode modified by Indigo Carmine	7.5	DPV	[9]
Phthalocyanine-based sensor	3.8	DPV	[26]
Gold ultra- microelectrode	16	LSV	[27]

The sensitivity of the sensor developed was compared to some sensors developed in the literature. The Table 3 presents the results of this investigation. All detection limits listed in the table are in the nanomol per liter (nmol/L) range.

The detection limits of this study are low compared to those reported in the literature. This sensitivity would be linked to the technique used and the electrode material. Anodic stripping voltammetry is an ultra-sensitive technique that can detect low concentrations of pollutants.

The Modifier incorporated into the carbon paste is a complex matrix made up of many chemical compounds that may have an affinity for complexing mercury

4. CONCLUSION

Electrochemical methods are well known as very powerful techniques for determination of diverse range of metallic and biological targets in environmental, biological and industrial samples. This work aims to provide a solution to the control of water quality in metal ions by detection in wastewater residues from artisanal mining. These activities release significant quantities of water into surface waters used for human consumption. Contamination of this water is not without consequences for humans. The objective of this study is therefore the implementation of a low-cost, but effective electrochemical sensor against mercury. To do this, *Moringa Oleifera* seed powder was incorporated into the carbon paste. The 10 % incorporation rate has been of most interest in cyclic voltammetry. The electrode thus developed was used in anodic stripping voltammetry to detect mercury. Analysis of the properties of this new CPEM-MO electrode showed that this electrode is seven times more

sensitive than bare carbon paste. Its detection limit is 0.4 nMol/L, lower than the authorized limit of 30 nMol/L by the World Health Organization (WHO). This sensor is of certain interest because it allows for routine monitoring of water and various matrices at reduced cost.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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