

Square wave voltammetry for electro catalytic detection of the antibacterial amoxicillin by a carbon paste electrode modified with zinc oxide nanoparticles ZnO@CPE. Analytical application: Milk, Human urine and Tap water

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ABSTRACT

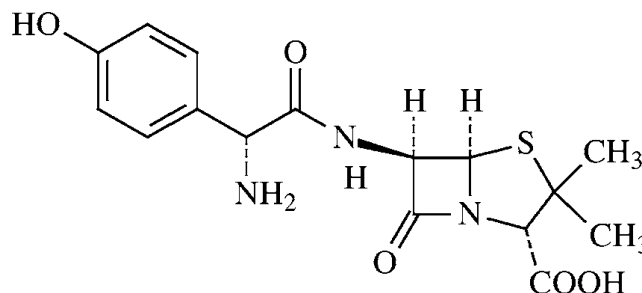
Amoxicillin is an antimicrobial of the penicillin class discovered in 1928, and is among the most widely used antibiotics to treat bacterial related infectious diseases, the purpose of this paper is to construct a simple platform for electrocatalytic detection of amoxicillin using a carbon paste electrode modified with zinc oxide nanoparticles. The ZnO@CPE modified electrode is constructed by simple and inexpensive processes using hydrothermal methods. Our results have shown that the ZnO/CPE modified electrode has excellent electrocatalytic activity compared to the unmodified CPE electrode with consistent, reproducible, stable behavior. The electrochemical behavior of the amoxicillin oxidation reaction is diffusion controlled and fully reversible. The effect of PBS pH on the antibacterial amoxicillin behavior shows that the number of protons and electrons was equal, The electrochemical parameters diffusion coefficient (D) and catalytic rate constant (k) were $1.135 \times 10^{-4} \text{ cm}^2/\text{s}$ and $8.314 \times 10^3 \text{ mol.l}^{-1}.\text{s}^{-1}$ respectively. The ZnO@CPE sensor was demonstrated a wide range of concentration linearity from 10^{-4} mol/l to 10^{-6} mol/l with electrocatalytic current, the detection limit was estimated $2.91 \times 10^{-7} \text{ mol/l}$ according to Square-wave voltammetry (SWV) method. The morphology and chemical composition of the constructed electrode paste were characterized by SEM and XRD suggesting the formation of nanocrystals of zinc oxides on the carbon sheets with the order size 22.95752 nm . The proposed sensor has been tested to determine the amoxicillin AMX in real samples.

Keywords: Amoxicillin, Square wave voltammetry, ZnO@CPE, electrocatalytic.

INTRODUCTION

Amoxicillin (AMX), (D- α -amino-p-hydroxybenzyl penicillin trihydrate) (Scheme 1) is a synthetic antibiotic derived from ampicillin [1]. AMX belongs to the spectrum of B-lactam antibiotics with a B-lactam-based formulation and has antibacterial activity with a structure that justifies its chemical and pharmacological properties. It is also easily adsorbed in living organisms and blocks peptidoglycan synthesis in bacterial

cells [2,3]. This facilitates its oral administration. It reaches active levels in the blood after one to two hours and is detectable after six hours of administration [4].



Scheme 1: Structure of amoxicillin.

Amoxicillin is an antibacterial agent widely used worldwide to treat various diseases, most commonly bacterial infections in humans and animals [5,6]. The widespread use of amoxicillin allows the transmission of trace amounts of amoxicillin metabolites in animal products such as milk and meat. In addition, the presence of the antibiotic amoxicillin in wastewater results in a very high disruption of non-target microorganisms living in the environment [7]. Due to the therapeutic relevance of the antibacterial amoxicillin, it is very important to develop a very sensitive and fast determination method with very low detection limits, in this perspective we chose to study the detection of amoxicillin in real samples using a carbon paste electrode modified with zinc oxide nanoparticles in order to improve the selectivity and sensitivity of the active surface of the working electrode.

Various methods have been reported in the literature for the determination of the active substance Amoxicillin, such as high performance liquid chromatography (HPLC-HP) [8-9-10-11] and flow injection analysis [12], spectrophotometry [13-14-15-16-17], liquid chromatography [18-19]. Fluorimetry [20], These methods, although very effective, and cost-effective. But they are expensive and are not available in most analytical laboratories. In recent years, electrochemical methods are widely applied. In the analysis of biological and environmental samples for the determination of electroactive species due to their rapidity, simplicity and low analytical cost. Several research works have been carried out on the modification of the working

electrode surface in order to obtain a high electrocatalytic activity for the detection of amoxicillin. For example, A. Hatamie and all others detected AMX using a ZnO NRs/gold/glass electrode note [21]. Rezaei and Damiri 2009 [22] ; Fouladgar et al 2011 [23] detected AMX by applying multiwall carbon nanotube (MWCNT) electrodes. Santos et al 2008 [24]; Uslu and Biryol 1999 [25]; Bergamini et al 2006 [26]; Ojani et al 2012 [27] adopted a modified carbon paste electrode.

In this research, we apply the integration of modified graphite carbons with ZnO microparticles to develop a novel electrochemical sensor with high electrocatalytic properties and support a large number of electrochemical measurements for the detection of amoxicillin antibiotics in real contaminant samples. The synthesis of zinc oxide microparticles was performed by thermal processes, the synthesis processes were studied and optimized. This focused project will provide a real analytical strategy for the electrocatalytic detection of amoxicillin in a linear detection range of 10^{-4} to 10^{-6} mol/l with a very low limit of detection (LOD) and quantification (LOD), and will also explain the electrochemical activities of amoxicillin molecules on the surface of our electrodes constructed of ZnO@CPE which is diffusion controlled. The chemical composition and surface morphology of the electrode constructed with modified graphite carbon pastes were studied by X-ray diffraction (XRD) and SEM techniques. The ZnO@CPEs showed strong catalytic activity to detect the antimicrobial amoxicillin in the presence of other organic substances, the Square-wave voltammetry (SWV) technique is applied to measure the intensity of the amoxicillin oxidation current at the surface of the ZnO@CPE electrode ; the results obtained reflect a proportionality between the concentration of amoxicillin and the measured electrocatalytic current. The proposed sensor has been successfully implemented for the detection of trace amounts of amoxicillin in real samples such as commercial milk, human urine and tap water with satisfactory results.

EXPERIMENTAL

Materials and methods

The 0.1 M phosphate buffer (PBS) was prepared by mixing NaH_2PO_4 (Fluka) and Na_2HPO_4 (Fluka). Sodium Acetate Buffer (ABS) (Fluka) - Acetic acid (Fluka) was prepared by combining acetic acid and sodium acetate solution. The active ingredient Amoxicillin (99%, analytical grade), zinc nitrate $\text{Zn}(\text{NO}_3)_2$ (98%, analytical grade) and carbon graphite (99% purity) were supplied by Sigma-Aldrich,

Potassium hexacyanoferrate III $\text{K}_3\text{Fe}(\text{CN})_6$ and potassium hexacyanoferrate IV $\text{K}_3\text{Fe}(\text{CN})_6$ were obtained from Fluka.

The active substance ascorbic acid (AA), uric acid (AU), Hydroxychloroquine (HCO) were purchased from Sigma-Aldrich

The Ph figures were adapted by concentrated solutions of H_2SO_4 (high analytical quality) or by solutions of NaOH. The different readings of the Ph-values were carried out by a PH-meter of analytical quality.

The different aqueous solutions were prepared with double distilled water.

Instrumentation

Potentialstat /galvanostat type Origalys ElectroChem SAS (France mode) driven by origamastre 5 software installed in a personal computer allows to process the acquired data, and electrochemical cell with 5 walls and three electrodes: a ZnO@CPE note electrode plays the role of working electrode, an Ag/AgCl reference electrode saturated by 0.3M KCl and a platinum auxiliary electrode to ensure the circulation of the electrocatalytic current between the auxiliary electrode and the working electrode

The JEOL SEM installed at the research unit of the University Ibn Zohr Agadir Morocco allows to visualize the images of all the pastes we prepared at different calcination temperatures. The X-ray spectra were performed with the X-ray diffractometer PERT-PRO(BRUKER-AXS) with CuK radiation ($\lambda=1.5406 \text{ \AA}$) installed at the research unit of the University IBN Zohr Agadir Morocco, allowing to characterize the chemical composition of the pastes we prepare. The diffraction angles (2θ) were between 10° and 100° with a step of 0.02° 2θ . The systronics Model-361 digital pH meter was used for pH measurements. All electrochemical experiments were performed in a conventional electrochemical cell with a three electrode system. Connected to the potentialstat/galvanostat and a computer to process all experimental data obtained.

Procedure

Operational conditions of electrochemical measurements

Electrochemical impedance spectroscopy was performed in a 1 M solution of $\text{K}_3[\text{Fe}(\text{CN})_6]/\text{K}_4[\text{Fe}(\text{CN})_6]$ containing 0.1 M KCl with a frequency range of 10 kHz to 100 Hz and an amplitude of 10 mv, cyclic voltammograms (CV) were performed in the potential range -200 to 1200 mv. Square-wave voltammetry (SWV) measurements were performed under optimized conditions with an applied potential between 00mv and 1000mv. Chronoamperometric curves were established using the ZnO@CPE electrode constructed with an applied potential of 600mv for a duration of 3 min. The surface morphology of the constructed modified electrode was analyzed by SEM, the chemical constitution of the modified pastes prepared to elaborate the ZnO@CPE electrode was characterized by X-ray diffraction (XRD), the diameters of the nanocrystals from the zinc oxide synthesized on graphite carbon sheets were evaluated using the Debye-Scherrer equation.

Preparation of ZnO@CPE

The adapted strategy for the development of the ZnO nanoparticle modified electrode is as follows: carbon-graphite and zinc nitrate were mixed at different ratios $\text{Zn}(\text{NO}_3)_2$ / GC: 5%, 10%, 15%, 20% and 25% in an agate mortar and ground for 20 minutes to obtain a functional electrode paste. Then, the obtained mixture was calcined at different calcination temperatures : 200°C , 300°C , 400°C , 500°C and 550°C in a programmable oven for 12 hours at constant speed. The

obtained paste was then inserted into the electrode cavity (fabricated in the laboratory with an exact geometric surface of 0.1256 cm^2) and smoothed. The electrical Step

- We optimized the rate of zinc nitrate $\text{Zn}(\text{NO}_3)_2$ and the amount of carbon graphite to obtain the best electrocatalytic performance for the oxidation of AMX molecules on the surface of the working electrode constructed.
- After optimization, the rate of zinc nitrate and the amount of carbon graphite. The different pastes prepared were calcined at different temperatures ranging from 200°C to 550°C in a programmable oven for 12 hours in order to optimize the optimal calcination temperature.

Analysis of real samples

Tablet assay procedure

Five pieces of the antibiotic amoxicillin were finely ground in a mortar for 20 minutes until a fine powder of amoxicillin was obtained. Then, a precise amount of the calculated amoxicillin corresponding to a concentration of 10^{-3} mol/l . The mass was transferred to a 200 ml flask containing a 0.1 M phosphate buffer solution (pH7), a suitable volume of 35 ml of the prepared solution was poured into an electrochemical cell, the cyclic voltammograms (CV) and Square-wave voltammetry (SWV) technique, were recorded using the electrode constructed. The low concentration solutions were prepared by precise dilution with PBS (pH7).

Lait commercial, l'urine humaine and l'eau du robinet assay procedure

We chose three real samples: Commercial milk, human urine and tap water to test the applicability of the constructed electrode.

The urine samples were obtained through a local clinic near IBN ZOHR Agadir MOROCCO University and stored in a refrigerator until they were analyzed. The tap water samples we examined were from our laboratory.

The urine samples were treated with 0.2 ml of methanol to decrease the protein level, after 15 minutes the samples were inserted in a centrifuge for 15 minutes at a speed of 4000 tr/min at room temperature in our laboratory, then the urine samples were filtered through a micropore $50\mu\text{m}$ filter paper. The resulting filtrates were spiked with a quantity of amoxicillin and transferred to a 100 ml solution of PBS (pH7). A volume of 40 ml of urine samples spiked with amoxicillin was transferred to an electrochemical cell, detection was performed using the modified constructed electrode, the recovery rate was determined by the standard addition method.

Liquid milk samples were purchased from a supermarket near the university, 5 ml of milk was measured and poured into a 100 ml flask, after which it was diluted with PBS pH7. Voltammograms were recorded, indicating the absence of observed peaks corresponding to amoxicillin oxidation. After this verification test, the milk samples were introduced into a centrifuge for 10 minutes at a speed of 4000 tr/min at room temperature in our laboratory. After filtration, the filtrate was contaminated with a quantity of amoxicillin taken.

Voltammograms were recorded by Square-wave voltammetry (SWV) method, the recovery rate was determined by standard addition method.

Tap water samples were used without any pretreatment.

RESULTS AND DISCUSSION

The effect of the supporting electrolyte used to detect AMX

The effect of the supporting electrolyte on the reversibility of l'amoxicillin was studied. The supporting electrolytes tested were PBS phosphate buffer, Britton Robinson buffer (BRS), sodium acetate buffer (ABS) and sulfuric acid buffer. The responses obtained (Figure.1) show that the oxidation and reduction peaks of l'amoxicillin are very sensitive to the nature of the electrolyte used. The signals are very weak and difficult to detect when using the sulfuric acid buffer, but with the sodium acetate buffer and the Britton Robinson buffer we observe that the signals of the l'amoxicillin peaks are less intense. On the contrary, the PBS phosphate support electrolyte allows us to give better responses in terms of current intensities of the l'amoxicillin oxidation and reduction peaks, reflecting that the PBS phosphate support electrolyte is very favorable for driving the -OH donor groups of the L'amoxicillin molecules to the surfaces of our electrode constructed for electrooxidation. Therefore, this support was chosen as the optimal support electrolyte for electrocatalytic detection of l'amoxicillin in our future work.

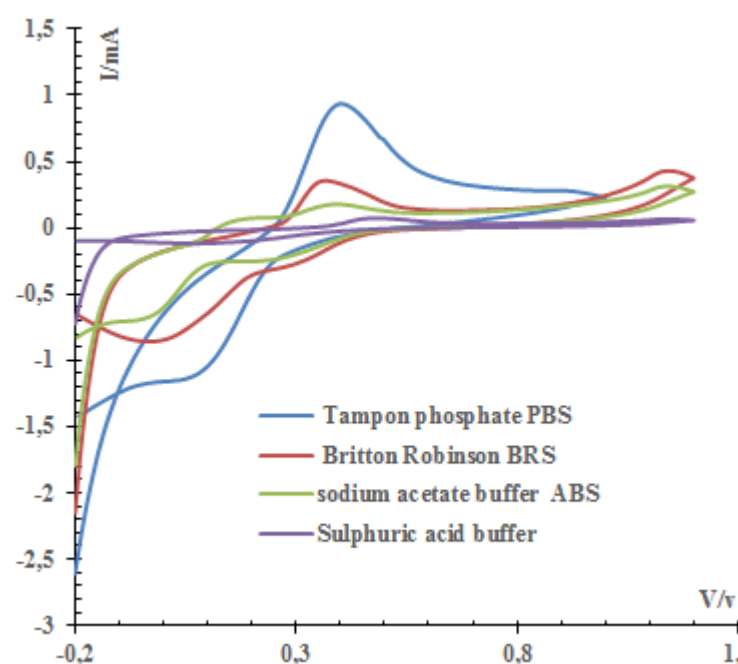


Figure1: The effect of the supported electrolyte tested for the detection of 10^{-3} amoxicillin by cyclic voltammetry in the potential range -200mv to 1200mv with a scan rate 20mv/s .

Then, we evaluated the effect of the concentration of the selected supporting electrolyte, the concentration ranges examined being 0.1 M, 0.2 M, 0.3 M and 0.4 M. According to the

results obtained, it can be seen that the best peak shift and current intensity were observed when using the supporting electrolyte at the concentration of 0.1 M.

Optimization of Zn (NO₃)₂/CG percentage and calcination temperature

Optimization of percentage the Zn (NO₃)₂

Figure 2. Shows the responses obtained, we notice that the best response was observed at 20% Zn(NO₃)₂ reflects that this percentage causes a large number of electro active sites on the surface of the working electrode which creates a very large porosity to the constructed ZnO@CPE. However, increasing the amount of modifier shifts the threshold value of the Amoxicillin oxidation. This generates a decrease in the sensitivity of the surface response of the constructed electrode. Therefore, the 20% Zn(NO₃)₂ modifier provides better electrocatalytic performance of our ZnO@CPE modified electrodes for the detection of Amoxicillin. This is a note for our future work.

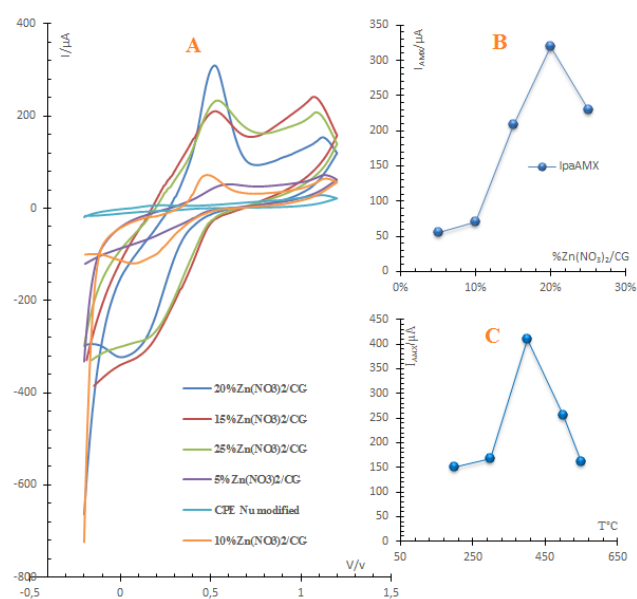


Figure 2: (A). Cyclic voltammograms of 10-4 M amoxicillin recorded in pH7 PBS solution on a modified carbon paste electrode constructed with different modification rates, in the imposed potential range of -200mV to 1200mV and a scan rate of 20mV/s. **(B)** .Variation of I_{pa} (AMX) as a function of modifier amount. **C** the variation of I_{pa} (AMX) as a function of calcination temperature.

Optimization of calcinations temperature.

The calcination temperature of the pastes we prepared is a primary factor in the field of nanoparticle syntheses. It can be seen that the best electrocatalytic performance of the constructed electrode (in terms of electrocatalytic current intensity and potential) was achieved for the pastes that were calcined at a temperature of 400°C (as seen in Figure 2.C). This shows that the size and number of zinc oxide nanoparticles synthesized on the carbon graphite support at this temperature

were very significant. This enhanced the electrocatalytic effect of the surface of our electrode constructed for the detection of amoxicillin antibiotic molecules. The responses obtained reflect the fundamental role of zinc oxide nanoparticles in the field of electro catalysis.

The ideal temperature of calcination was thus fixed around 400°C for the various pastes of our constructed electrode proposed thereafter.

Characterization of ZnO dispersed in CPE.

X-ray diffraction (XRD) characterization of ZnO@CPE

Figure 6. Show the X-ray diffraction spectra registered on the prepared ZnO@CPE paste calcined at 400°C and the graphite carbon paste.

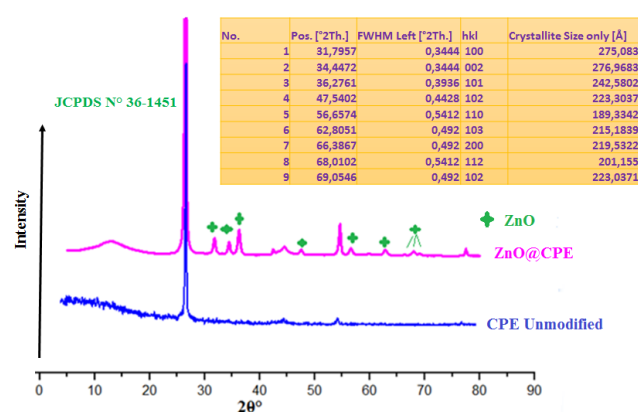


Figure 3: DRX of CPE and ZnO@CPE calined at temperatures 400°C

The comparison between the two spectra one noting the appearance of the new site peaks in the positions at 2θ = 31.720°, 34.320°, 36.120°, 47.360°, 56.520°, 62.880°, 69.2°, 70.3° and 70.9°. Which correspond respectively to the crystal planes (100), (002), (101), (102), (110), (103), (200), (112) and (102) corresponding to Zinc oxide nanoparticles the file (Joint Committee on Powder Diffraction Standards (JCPDS) card JCPDS No. 36-1451) confirming the results obtained [28]. The nanocrystals of Zinc oxides are crystallized in the hexagon system with lattice parameters a=b= 3.234 Å and c= 5.186 Å.

We also note that the peak at position 36.2761° is very intense which corresponds to the (111) plane. Suggested that the formation of the nano crystals of Zinc oxides on the carbon sheets of graphite is done according to the {(hkl)=(101)} plane.

The size of the average crystallite has been evaluated using the Debye-Scherrer formula [29] relating the width of the diffraction peaks to the size of the crystallites. This formula is given by the following equation.

$$D = \frac{0.89\lambda}{\beta \cos \theta}$$

With D is the average crystallite size (nm), λ is the wavelength ($\text{\AA}$) of the X-rays used, β is the angular width at half height of the peak of (rad), and θ is the diffraction angle that corresponds to the maximum intensity of the peak.

According to the results shown in the table below the average size of the zinc oxide nanoparticles was 22.95752nm.

Morphological characterization of ZnO@CPE

The images by scanning electron microscope were carried out using a microscope type JEOL JSM 6830 equipped with an EDX analyzer (Energy dispersive X-ray analysis) allowing the analysis of the elemental chemical composition of our paste of the constructed modified electrode.

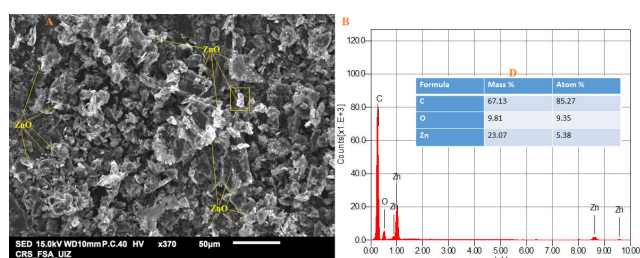


Figure4: SEM image of ZnO@CPE (A), EDX (B), atomic and mass ratio of Zn, O and C elements (C).

The analysis of the acquired image shows that the surface of the modified carbon paste consists of zinc oxide particles in the form of aggregates and polyaggregates dispersed over the entire surface of the graphite carbon paste with different shapes and sizes of the order of very varied. This phenomenon is probably explained by the speed of calcination which leads to the formation of aggregates and polyaggregates. Let us also note the presence of a very large number of ZnO particles with diameters in the micrometer and nanometer range over the entire surface which extends the active surface of the working electrode and generates a very high electrocatalytic performance compared to the unmodified carbon paste electrode.

Figure.4. B. Shows the elemental chemical composition analysis of the prepared modified paste calcined at the optimum temperature of 400°C by EDX (energy dispersive X-ray analysis). The analysis spectrum shows the presence of four peaks at the positions of 0.9 Kev; 1 Kev; 8.6 Kev and 9. Kev which characterize the chemical element Zn and a peak at the position of 0.5Kev which characterizes the element of oxygen; the peak which is the most intense characterizes the element of carbon. These results attest that the carbon material is successfully doped with the elements Zn and O. The analysis of the mass and atomic percentages of the different elements confirms this. They clearly indicate a very significant rate of the elements of Zinc and Oxygen in the electrode paste constructed in figure 4.D.

Finally. Structural and morphological studies of the prepared modified graphite carbon paste attest to the synthesis of zinc oxide nanocrystals on the graphite carbon sheets which can lead to the obtention of an electrochemical sensor capable of detecting the antimicrobial molecule Amoxicillin.

Electro-catalytic behavior of amoxicillin at ZnO@CPE modified and CPE non modifiée

The electrocatalytic performance of the ZnO@CPE constructed electrode compared to the unmodified CPE were examined using the cyclic voltameter and SWV.

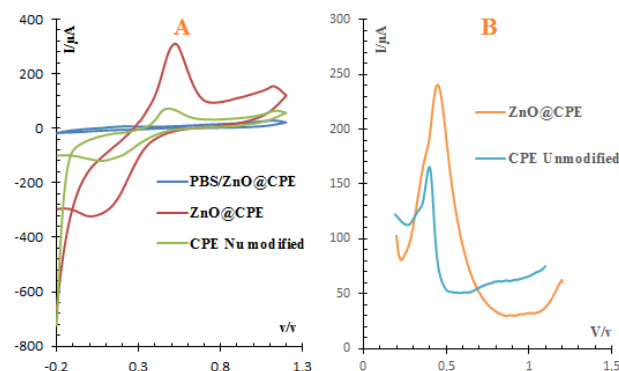


Figure 5: (A) CVs of 10-3 mol/l AMX in 0.1M PBS (pH7) for unmodified CPE and ZnO@CPE. (B) SWVs of 10-3 mol/l AMX in 0.1M PBS (pH7) for unmodified CPE and ZnO@CPE.

Experimental cyclic voltammograms obtained from 10-3 M amoxicillin with 0. 1M PBS(pH7) and scan rate ($v = 20\text{mv/s}$) on unmodified CPE and ZnO@CPE (Figure.5 .A). Have shown that the electrochemical processes of the redox reaction of amoxicillin are reversible. And also show that the electrocatalytic current intensity of the anodic and cathodic peaks and are significantly increased compared to the ZnO@CPE electrode. This result reflects that the presence of ZnO nanoparticles in the surface of the working electrode has improved the electrocatalytic performance for amoxicillin analysis.

Therefore, the number of amoxicillin molecules oriented toward the surface of the ZnO@CPE electrode is very large during the potential sweep from -200mv to 1200mv compared to that oriented toward the unmodified CPE electrode.

Figure 5..B. Shows the square voltammograms obtained from 10-3 M amoxicillin and 0. 1M PBS (pH7) on the ZnO@CPE electrode and the unmodified CPE electrode (Figure 8.B) under the following conditions (scan rate 20mv/s, pulse amplitude = 60 mV, $t_{acc} = 60\text{s}$, ΔE increment = 10mV $E_{acc} = 550\text{mV}$, frequency $f = 80\text{ Hz}$). The results clearly indicate that the peak oxidation current of amoxicillin was very high on the ZnO@CPE electrode compared to the unmodified CPE electrode and the peak oxidation potential is slightly shifted to very positive values, this improvement attests that the zinc oxide nanoparticles synthesized on the graphite carbon sheets created a very high electrocatalytic effect on the working electrode surface. This increases the chances of attracting a significant number of amoxicillin molecules to the surface of the working electrode we constructed.

Comparison between ZnO@CPE and CPE by using cyclic voltammetry

The electrochemical behavior of $\text{Fe}(\text{CN})_6^{3-/4-}$ couples was evaluated on the unmodified CPE and ZnO@CPE electrode constructed in the presence of 10^{-3} M $[\text{Fe}(\text{CN})_6]^{3-/4-}$ and 10^{-1} mol/l KCl with a scan rate of 20 mv/s by applying the cyclic voltmeter.

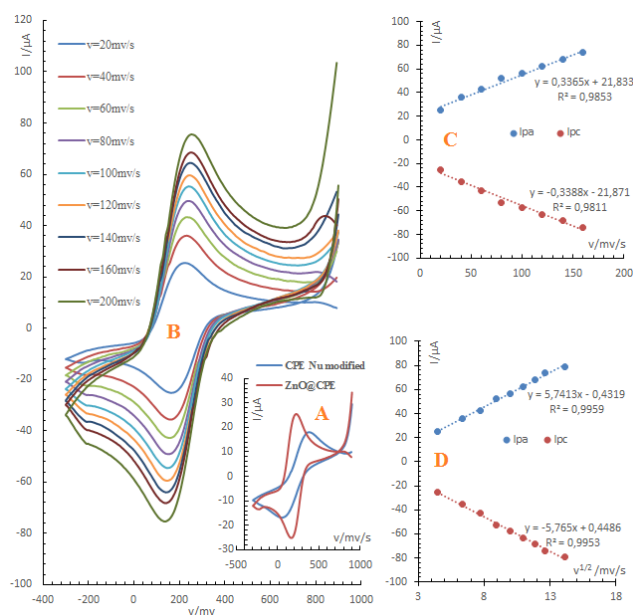


Figure 6 .(A). CVs obtenus pour CPE unmodifié et ZnO@CPE en présence de 1 mM $[\text{Fe}(\text{CN})_6]^{3-/4-}$ contenant une solution de KCl 0,1 M à une vitesse de balayage de 20 mV·s⁻¹. CVs . Shows the cyclic voltammograms recorded with the ZnO@CPE electrode at different scan rates ranging from 20mv/s to 200mv/s (B). (C) Variation of I_{pa} and I_{pc} with scan rate. (D) Variation of I_{pa} and I_{pc} with the square root of scan rate

Figure.6. A. Shows the cyclic voltammograms obtained on the unmodified CPE electrode and the modified ZnO&CPE electrode. The comparison between the two cyclic voltammograms indicates that the $[\text{Fe}(\text{CN})_6]^{3-/4-}$ redox system is fully reversible with ΔE_{ac} (CPE) = 310 mv which means that the electron transfer is very slow. On the modified ZnO@CPE electrode, a significant increase in the current intensity of the anodic and cathodic peaks of the $[\text{Fe}(\text{CN})_6]^{3-/4-}$ system is observed almost twice as high as that obtained on the unmodified CPE electrode. It is also observed that the potential difference between the anodic and cathodic peaks is strongly decreased such that ΔE_{ac} (ZnO@CPE) = 60 mv. This result will reflect well that the electronic conductivity of the modified working electrode surface is very high which will generate a very fast electron transfer. Therefore, the presence of zinc oxide microparticles on the graphite carbon sheets will form highly conductive bridges that will increase the electrostatic attraction at the interface of the ZnO@CPE supported electrolyte electrode surface - in which we have the molecules of the ferrocyanide system $[\text{Fe}(\text{CN})_6]^{3-/4-}$.

Figure.6.B. Shows the cyclic voltammograms recorded with the ZnO@CPE electrode at different scan rates ranging from

20mv/s to 200mv/s. Figures 6.C and 6.D depict the variation of the anodic and cathodic current intensity of the $[\text{Fe}(\text{CN})_6]^{3-/4-}$ system as a function of scan rate and root scan rate respectively. The results suggest good linearity which reflects that the process of the $[\text{Fe}(\text{CN})_6]^{3-/4-}$ system is diffusion controlled. Therefore. The placement of ZnO particles on the surface of the working electrode generates a highly conductive surface that promotes redox reactions of the ferrocyanide.

Electrochemical impedance spectroscopic studies

The Nyquist curves obtained for the two unmodified CPE and modified ZnO@CPE electrodes are shown in Figure 7.

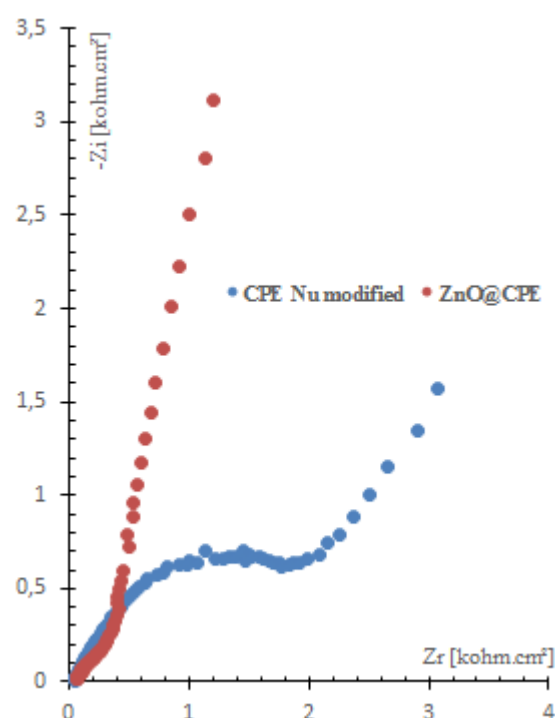


Figure7. Nyquist EIS obtenu pour le CPE Nu et ZnO@CPE en présence d'une solution de 1Mm de $[\text{Fe}(\text{CN})_6]^{3-/4-}$ contenant 0,1 M de KCl, amplitude 10mv, gamme de fréquence 10KHz à 100Hz.

The comparison between the two Nyquist reflects that on the bare CPE electrode there is a semicircle in the high frequency range characteristic of the charge transfer process and its diameter is equal to the value of the charge transfer resistance $R_{ct}(\text{CPE}) = 2 \text{ Kohm/cm}^2$. Reflecting that the kinetics of the electron transfer of the $[\text{Fe}(\text{CN})_6]^{3-/4-}$ system at the interface of the unmodified CPE electrode is slow, this translates into the fact that the electrostatic attraction at the interface of the CPE electrode surface is very blocked. Followed by a straight line in the low frequencies which corresponds to the transfer by diffusion. But after its modification by 20% modifier used we observe a noticeable decrease of the semicircle in the high frequency range with a very small diameter which means a strong decrease of the charge transfer resistance such that $R_{ct}(\text{ZnO@CPE}) = 0.2 \text{ Kohm/cm}^2$. Reflecting that the electron

transfer kinetics is very fast which means a strong electrostatic attraction between the surface of the modified ZnO@CPE electrode and the negatively charged [Fe(CN)₆]^{3-/4-} system molecules, then a straight line in the low frequency characteristic of a diffusion process. This result obtained demonstrates the role of the zinc oxide microparticles synthesized on the graphite carbon sheets in accelerating the electron transfer rate on the surface of the ZnO@CPE electrode.

According to the electrochemical studies by EIS and CVS carried out, it can be said that the zinc oxide nanoparticles increase the signal intensity of the recorded electrocatalytic current and also cause a significant improvement in the intensity ratio of the current/noise recorded during the specific recognition of the antimicrobial amoxicillin on the surface of the working electrode, all this due to the increase in the electrocatalytic surface/volume ratio.

Effect of scan rate

The electrochemical behavior of the antibiotic amoxicillin was evaluated on the electrocatalytic surface of the electrode constructed 20% ZnO@CPE in the scan rate range from 3mv/s to 50mv/s using cyclic voltmeter techniques in the potential range between -200mv and 1200mv with 10⁻⁴ M AMX in 0.1 M PBS (pH7).

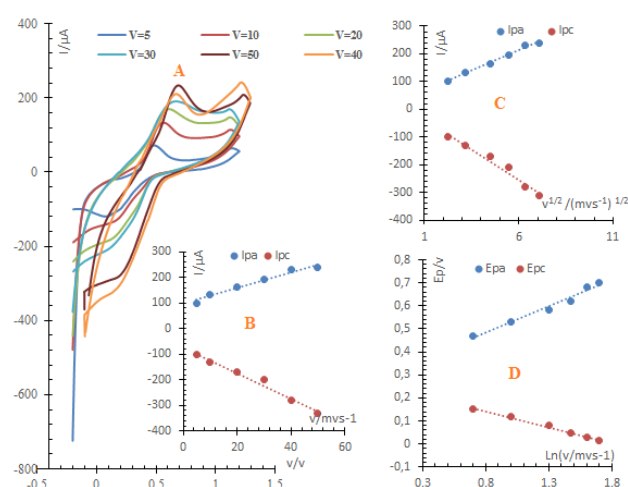


Figure 8: (A) CVs of 10⁻³ mol/l AMX in 0.1M of PBS (Ph7). (B) Variation of I_{pa} and I_{pc} with scan rate. (C) Variation of I_{pa} and I_{pc} with the square root of scan rate. (D) variation of E_{pa} and E_{pc} with Ln (v).

We note that the cyclic scan voltammograms in the range of potential -200mv and 1200mv obtained (Fig.8.A). Clearly indicating that the peaks of oxidation/reduction of amoxicillin molecules are very sensitive to the effect of potential sweep rate with $\Delta E = E_{pa} - E_{pc} = 49 \text{ mV}$ / ZnO@CPE vs AgCl and $|I_{pa}/I_{pc}| = 1$ which suggests that the electrochemical behavior of the reaction of amoxicillin on the surface of the modified electrode constructed is completely reversible [30].

Fig.8.B. Show the electrocatalytic current intensity of the anodic and cathodic peaks corresponding to the oxidation and reduction of AMX on the modified ZnO@CPE electrode were varied linearly with increasing scan rate with regression

equations $I_{pa}(\text{AMX}) = 3.0937 v + 97.079$ ($R^2 = 0.9795$) and $I_{pc}(\text{AMX}) = -5.0027 v - 73.096$ ($R^2 = 0.9895$) respectively.

Similarly, the current intensity of the anodic and cathodic peaks are found to vary linearly with the square root of the scan rate in Figure.8.C. According to the following regression equations, $I_{pa}(\text{AMX}/\text{ZnO@CPE}) = 29.329 v^{1/2} + 36.497$ ($R^2 = 0.9914$) and $I_{pc}(\text{AMX}/\text{ZnO@CPE}) = -43.734 v^{1/2} + 9.1782$ ($R^2 = 0.9763$) respectively. These results reflect that the electrocatalytic reaction process of amoxicillin on the surface of our constructed sensor is controlled by diffusion phenomena.

Meanwhile, the evolution of the anodic and cathodic peak potentials of L'amoxicillin with the logarithm of the scan rate Ln(v) is presented in Figure .8.D. It is clear that the values of the peak potentials vary linearly with the logarithm of the scan rate Ln(v), with a regression equation $E_{pa}(\text{AMX}) = 0.02274 \ln(v) + 0.3019$ ($R^2 = 0.9732$) and $E_{pc}(\text{AMX}) = 0.01372 \ln(v) + 0.252$ ($R^2 = 0.9909$) with a correlation coefficient ($R^2 = 0.9984$) respectively. This indicates that the potentials of the electrocatalytic reaction peaks of L'amoxicillin on the surface of the electrode modified with zinc oxide nanoparticle arrays are very sensitive to the scan rate of electron transfer. Laviron showed that the equation between E_p and LnV when a system is reversible (equations 2 and 3) are [31].

$$\text{Eq2 : } E_{pa}(\text{AMX}) = E^\circ - \frac{RT}{(1-\alpha)nF} \ln \frac{(1-\alpha)nF}{kRT} - \frac{RT}{(1-\alpha)nF} \ln V$$

$$S_{pa} = \frac{RT}{(1-\alpha)nF}$$

$$\text{Eq3 : } E_{pc}(\text{AMX}) = E^\circ - \frac{RT}{\alpha nF} \ln \frac{\alpha nF}{kRT} - \frac{RT}{\alpha nF} \ln V$$

$$S_{pc} = -\frac{RT}{\alpha nF}$$

The ratio $S_{pa}/S_{pc} = \alpha/(1-\alpha) = 1.656$ to calculate the value of the transfer coefficient α was estimated to be 0.39. As a result. The number of free electrons in the electrooxidation reaction of amoxicillin molecules captured at the surface of the ZnO@CPE electrode is equal to $n=0.87$ ($n \approx 1$). This result is similar to the work done by J.A.Harrison et al [32].

$$\log K_s = \alpha \log(1-\alpha) + \alpha(1-\alpha) \log(\alpha) - \log\left(\frac{RT}{nFV}\right) - \frac{\alpha(1-\alpha)nF\Delta E_p}{2,3RT}$$

Avec F (C/mol) is Faraday's constant. R (J/(mol.K)) is the gas constant. T (K) . is the absolute temperature. α is the electron transfer coefficient

The responses obtained show that the value of the constant K_s has been estimated at 0.565 s⁻¹ for a rate of 10mv/s. Thus, the numerical value obtained is very close to the results reported in the literature by the work done by [33-34] . Therefore, this result suggests that the modified ZnO@CPE electrode has a higher electrocatalytic capacity to detect amoxicillin molecules in real samples.

Effect of pH

Several tests were performed with different pH values ranging from 3 to 8.5 using CV between -200 mV and 1200 mV potential with a scan rate of 20 mV/s. to study the effect of Ph of PBS buffer on the electrochemical behavior of AMX.

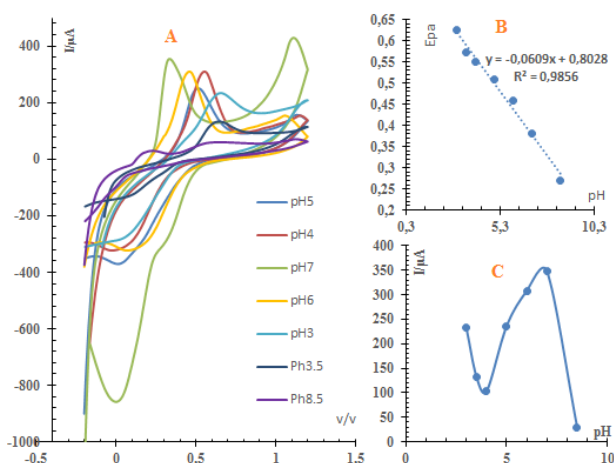


Figure 9: (A) CVs (at 20 mV/s) of 1.0×10^{-3} mol.L⁻¹ de AMX at different values of (pH= 3 ;3.5 ;4 ;5 ;6 ;7 ;8.5). Inset: (B) Plot of Epa vs pH and (C) Ipa vs pH.

The voltammograms obtained show that the redox potential of amoxicillin is very sensitive to the pH values of the PBS used (Fig.9.A). It can be noticed that the peak of the oxidation potential of amoxicillin is progressively shifted towards negative values when the pH increases. The linearity between Epa and the PBS pH values was expressed by the following equation $E_{pa} = -0.0609\text{pH} + 0.8028$ with a coefficient $R^2 = 0.9856$. The comparison between the obtained slope and the theoretical equations (4) of Nersis : we notice that the number of proton(m) and electrons(n) exchanged at the electroactive surface during the diffusion of amoxicillin molecules on our ZnO@CPE electrode were equal ($m/n = 1$). This result is comparable to the results of work conducted by [35] and [36].

$$\text{Eq 4: } E_{pc} = E^\circ - \left(\frac{2,303mRT}{nF} \right) \text{pH} = E^\circ - 0,059 \frac{m}{n} \text{pH}$$

The variation of the anodic oxidation current of amoxicillin as a function of Ph values reveals that the maximum electrocatalytic current was obtained in a neutral medium of pH7 (Fig.9. .B). This result leads to the conclusion that the neutral medium is a very favorable environment for the diffusion and orientation of the phenol group ($\phi\text{-OH}$) [37] from the side chain of the amoxicillin molecules to the active surface of the ZnO@CPE electrode. Therefore, pH 7.0 was chosen as the optimal pH for further experiments.

According to all the mentioned results, the most explanatory mechanism of electro oxidation reaction of amoxicillin on the electro catalytic surface of ZnO@CPE electrode is described in the scheme 2. This mechanism is similar to the findings of the work done by R. Ojani et al [38] and Samaneh Zeinalia et al [39].

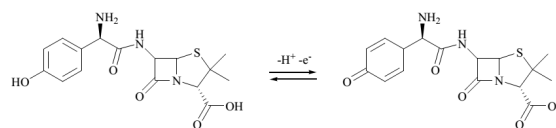


Figure 10: the explanatory mechanism of the electro-oxidation reaction of AMX on ZnO@CPE

Chronoamperometric studies

The results obtained for different concentrations of amoxicillin ranging from 10^{-5} to 10^{-3} mol/l obtained by the chronoamperometry technique were shown in Figure xxx. The obtained Cottrell chronoamperometry curves were examined in 0.1 M phosphate buffer (pH 7) with an applied potential of 450mV against the Ag/AgCl reference electrode for a duration of 3 minutes with the constructed ZnO@CPE modified electrode.

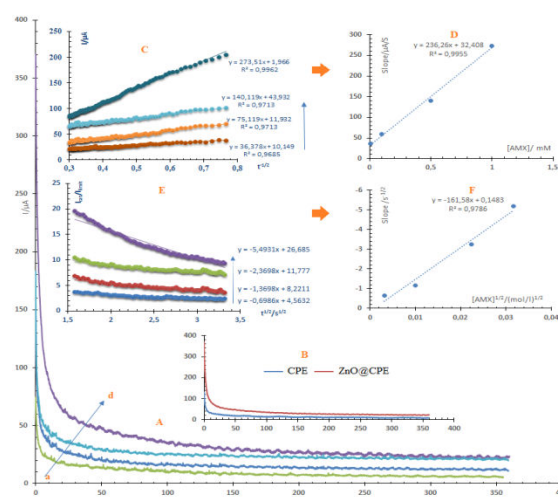


Figure 11: A Chronoamperograms of oxidation amoxicilline (450mV) for different concentrations within the range 10^{-5} M (a), 10^{-4} M (b), 5×10^{-4} M (c) and 10^{-3} M (d), at ZnO@CPE and calibration curve as figured in insert. (B) comparison entre l'effet électro catalytique entre ZnO@CPE et CPE .(C) plots I vs $t^{-1/2}$ obtained from Chronoamperograms (a) and (d), (D) plots of slopes lines against oxidation AMX concentration. (E) I_{cat}/I_{lim} vs $t^{1/2}$ and (F) plots of slopes lines obtained vs racine the concentration AMX

The diffusion coefficient D of the amoxicillin reaction on the ZnO@CPE surface was calculated from Cottrell's equation 5 [40, 41].

$$\text{Eq 5: } I = \frac{nFAC_{AMX}D^{1/2}}{\pi^{1/2}t^{1/2}}$$

Where n is the number of electrons transferred in electrochemical reaction, F(96,500 C mol⁻¹) is the Faraday constant (C/mol), A (0.1256 cm²) is the electrode area(cm²), C and D are the bulk concentration (mol/l) and the diffusion coefficient (cm²s⁻¹) of amoxicilline respectively .

We plotted the variation of the AMX electrocatalytic current as a function of $t^{1/2}$ (Figure 11.C) for the different Cottrell curves (a-d) obtained. Then, we plotted the different slopes of the straight lines obtained with their concentrations of amoxicillin (Figure 11.D). Thus, from the Cottrell equation mentioned above, we calculated the DAMX diffusion coefficient of the reaction amoxicillin was estimated to be $1.135 \times 10^{-4} \text{ cm}^2/\text{s}$.

The electrocatalytic rate constant K of electro-oxidation of AMX was evaluated using the following equation (6) [42 43 -44].

$$\text{Eq 6} \quad : \frac{I_{\text{cat}}(\text{AMX})}{I_{\text{limite}}} = \gamma^{1/2} [\pi^{1/2} \text{erf}(\gamma^{1/2}) + \exp(-\gamma/\gamma^{1/2})]$$

$I_{\text{cat}}(\text{AMX})$ and I_{limite} are the catalytic and limiting currents of ZnO@CPE fabriqué in the presence of AMX.

$\gamma = k_{\text{cat}} \text{CAMX} t$ avec k_{cat} est la constant de vitesse électrocatalytique, CAMX est la concentration de AMX en mol/cm^3 , t est temps en S.

Equation (6) can be simplified like this equation (7).

$$\text{Eq 7} \quad : \frac{I_{\text{cat}}(\text{AMX})}{I_{\text{limite}}} = \gamma^{1/2} \pi^{1/2} = (\pi k_{\text{cat}} \text{CAMX} t)^{1/2}$$

The variation of $I_{\text{cat}}/I_{\text{L}}$ as a function of square root of time ($t^{1/2}$) were plotted (figure 11.E), then we plotted the slope of the straight lines obtained with the square root of concentration of amoxicillin (figure 11.F). The value of the electrocatalytic rate constant k_{cat} was found to be $8.314 \times 10^3 \text{ mol.l}^{-1} \cdot \text{s}^{-1}$.

Square-wave voltammetry detection of amoxicillin

The calibration curve was established under the optimized conditions (Table 1), applying Square-wave voltammetry (SWV). The peak current intensity of the electrocatalytic oxidation changes with the concentration of amoxicillin in the range of 10^{-4} mol/l to 10^{-6} mol/l .

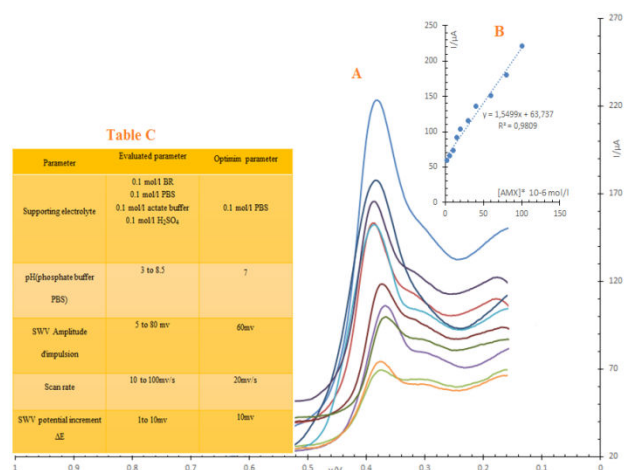


Figure 12: SWV of AMS in 0.1mol/l PBS (pH7) (A), Inset: plots of electrocatalytic peak current as a function of AMX

concentration (B), And Table (C) Value of the experimental variables optimum involved in the SWV.

The peak current intensity of the observed electrocatalytic oxidation of amoxicillin is proportional to its concentration in the whole studied range 10^{-4} mol/l to 10^{-6} mol/l . The equation of the calibration curve obtained is linear $I(\text{AMX}) = 1.5499[\text{AMX}] + 63.737$ with a coefficient $R^2 = 0.9809$.

The limits of detection (LOD) and quantification (LOQ) were calculated using the equations $\text{LOD} = 3 S_d / m$ and $\text{LOQ} = 10 S_d / m$ with m : the slope of the calibration line and S_d is the standard deviation of the blank response which was obtained from 8 repeated measurements of the blank PBS buffer solution.

$$\text{LOD} = 2.9110^{-7} \text{ mol/l}$$

$$\text{LOQ} = 9.72 \times 10^{-7} \text{ mol/l}$$

The performance of our ZnO@CPE constructed electrode was compared with some modified electrodes cited in the literature for the detection of amoxicillin under similar conditions. We have summarized the comparison in Table 1.

Electrod	Technique	Linear range μM	LOD μM	Ref
MWCNTs modified-GCE	CV	0.6-80	0.2	45
Modified CPE	CV	10-200	0.81	46
DMBQ/ZnO/CNTs/CPE	SWV		0.5	47
Modified CPE	SWV	Feb-25	0.92	48
Modified CPE	SWV	18.9-91.9	8.49	49
PGA/3D-GE/GCE	SWV	Feb-60	0.118	50
Ni-Curcumin /CPE	AMP	8-100	5	51
B-Diamond	DPV	0.5-40	0.25	52
	LC-MS/MS	0.014-54.7	0.014	53
	Spectrophotometry	13.6-82.0		54
	Chromatography	0.04-1.36	0.04	55
Cu(II)-NCL-CPE	SWV	0.04-100	0.02	56
FeCr2O4-MWCNTs/GCE	DPV	0.1-70	0.05	57
ZnO@CPE	Swv	100 – 1	0.291	This work

Table 1: Performance comparison of our ZnO@CPE constructed electrode with different modified electrode developed and different technique in the literatures.

LOD: limit of detection, CPE: Carbon Paste Electrode, GCE: glass carbon electrode CV: Cyclic voltammetry, DPV: differential

pulse voltammetry .SWV :square-wave voltammetry,AMP: amperometric

Response of the ZnO@CPE sensor in the presence of interferents

The selectivity of our developed ZnO@CPE electrodes for the detection of amoxicillin was investigated by plotting voltammograms in PBS phosphate buffer (pH 7) containing different organic chemical interferents: ascorbic acid (AA), uric acid (UA), Hydroxychloroquine (HCQ), with concentrations above the 100 μ M concentration of amoxicillin. The interferents examined are electro active in the detection potential range of amoxicillin. Their response is then compared with that observed at the same concentration of amoxicillin alone. The calculated recovery rate is very satisfactory, which means that the presence of interferents with a higher concentration than amoxicillin does not affect the detection of amoxicillin

Reproducibility and stability of the ZnO@CPE sensor response

The reproducibility of the electrode constructed of ZnO@CPE for the detection of the active substance amoxicillin was verified for three modified electrodes prepared separately, under the same processing and manufacturing conditions.

The responses provided reveal that the differences between the responses of the three electrodes are very small, which means that the method of preparation of the sensor in ZnO@CPE gives a fairly satisfactory reproducibility. And also The reproducibility of the response of the same electrode is characterized by a relative standard deviation (RSD) of 5.4% recorded over 10 consecutive measured for 1 mM amoxicillin, which demonstrates the reproducibility of the sensor for the detection of amoxicillin is quite acceptable.

The stability of our ZnO@CPE constructed sensor was evaluated against its implantation with amoxicillin (100 μ M) immediately after processing and fabrication, after storing the electrode for 25 days at room temperature in phosphate buffer solution at pH 7. A residual response of 91% of the maximum response was found.

Determination of antibacterial amoxicillin in real samples

The analytical performance of our electrode is tested for the detection of the antibacterial amoxicillin by the proposed ZnO@CPE electrode in real samples: commercial milk, human urine and tap water.

The Square-wave voltammetry (SWV) method was chosen to determine the antibacterial amoxicillin in the three selected samples, contaminated with adequate amounts of amoxicillin on the electrocatalytic surface of the fabricated electrode. The electroanalytical results are presented in Table 2. The relative standard deviations were less than 2.9% (n = 4),

Based on the results achieved, the calculated recoveries shown in Table 2. Reveal that the proposed sensor can be

successfully adapted to detect traces of the antibiotic amoxicillin in real samples such as commercial milk, human urine and tap water.

Sample	Spiked(μ M)	Found(μ M)	Accuracy %	RSD%(n=3)
Urine +AMX				
	50	49,68	97,13	1,22
	10	9,44	97,91	3,83
Tap water +AMX	1	0,95	96	1,34
	50	47,91	98,63	2,15
	10	9,93	97,6	3,33
	1	0,971	97,1	4,09
	50	47,73	95,46	2,36
	10	9,76	97,6	3,4
	1	0,946	94,6	2,98

Table 2: The relative standard deviations were less than 2.9% (n = 4),

CONCLUSION

In this study, we successfully constructed a graphite carbon paste decorated with zinc oxide nanocrystals synthesized on graphite carbon sheets by thermal process with sizes in the nanometer range. The ZnO@CPE sensor could be used as a suitable electrochemical sensor to detect antibacterial amoxicillin in real samples. XRD and SEM analysis confirmed the presence of zinc oxide nanoparticles. The electrochemical behavior of antibacterial amoxicillin on the ZnO@CPE electrode is fully reversible and their transfer to the electroactive surface of the constructed electrode is controlled by diffusion processes. The study of the pH effect of the phosphate buffer solution on the behavior of amoxicillin reflects that the number of electrons and protons are equal. The EIS study indicates that the charge transfer resistance of our electrode is very low compared to that observed on the unmodified carbon electrode. Chronoamperometric studies reveal that the diffusion coefficient D and electrocatalytic rate constant kcat for the electrocatalytic oxidation of antibacterial amoxicillin are 1.135 10^{-4} cm²/s and 8.314 10^3 mol.l⁻¹.s⁻¹, respectively. The analytical characteristics of ZnO@CPE indicate very acceptable reproducibility and selectivity with very broad stability. The ZnO@CPE sensor showed a very wide detection range of amoxicillin from 10⁻⁴ mol/l to 10⁻⁶ mol/l with a very low detection limit of 2.91 10^{-7} mol/l using the square wave voltammetry (SWV) method. The developed sensor has been successfully tested for the detection of amoxicillin in real samples, liquid milk, human urine and tap water with very satisfactory responses.

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