

ELECTROCHEMICAL SENSOR BASED ON ZNO-MODIFIED CARBON PASTE FOR SENSITIVE DETECTION OF TETRACYCLINE

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Abstract

In this paper, is studied the feasibility of preparing an electrochemical sensor based on carbon paste to determine one of the most commonly used antibiotics in our country, such as Tetracycline (TET). Various environmental and health agencies around the world emphasize moderate uses of these medications since they are often abused and can be disposed of indiscriminately with other untreated waste, becoming a potential for pollution of habitats or waters through which they may pass. The prepared sensor is based on carbon paste modified with 20% ZnO and after optimizing of indifferent electrolyte, pH and working conditions of the electrochemical techniques used, the detection limit for the target analyte TET was calculated from the calibration curve with the SWV technique, which resulted in about 4.5 ppm with RSD 9%, with a sensitivity of the method approximately 8 $\mu\text{A/ppm}$ and a correlation above 0.99 for the linear range 3-18 ppm. Morphological characterization of the modified carbon paste with SEM and EDX, provide an overview of the distribution of the modifier in the paste and the sizes that correspond to nm dimensions.

Key words: tetracycline, carbon paste, ZnO nanoparticles.

Përmbledhje:

Në këtë punim, studiohet munësia e përgatitjes të një sensori elektrokimik, bazuar në pasta karboni për të përcaktuar një nga antibiotikët më të përdorshëm në vendin tonë, por jo vetëm, sic është Tetraciklina (TET). Agjensit të ndryshme mjedisore dhe shëndetsore në mbarë botën, vendosin theksin në përdorime të moderuara të këtyre mendikamendteve pasi shpesh

abuzohet me to dhe mund të hidhen pakriter bashkë me mbetjet e tjera të cilat nuk trajtohen, duke u bërë potencial për ndotje të habitateve apo ujërave nëpër të cilat ato mund të kalojnë.

Sensori i përgatitur bazohet në pastë karboni të modifikuar 20 % me ZnO dhe pasi janë optimizuar të gjitha kushtet e punës si elektroliti indiferent, pH i tij dhe kushtet e punës së teknikave elektrokimike të përdorura u llogarit nga lakorja e kalibrimit me teknikën SWV, kufiri i diktimit për analitin target TET, i cili rezultoi rreth 4.5 ppm me RSD 9%, me një ndjeshmëri të metodës afërsisht 8 $\mu\text{A/ppm}$ dhe koeficienti i korrelacionit më i madh se 0.99 zonën lineare të punës 3-18 ppm. Karakterizimi morfologjik i pastës së karbonit të modifikuar me SEM dhe EDXs, japin një pasqyrë të shpërndarjes së modifikuesit në pastë dhe përmasave të cilat i përgjigjen dimensioneve nm.

Fjalë kyçe: tetraciklinë, pastë karboni, nanogrimca ZnO.

1. Introduction

Tetracycline (TET) determination and antibiotics in general, is essential for safeguarding public health, preventing antibiotic resistance, ensuring food safety and maintaining environmental quality. Tetracycline is commonly used in livestock to prevent or treat bacterial infections, (Zelalem Bitew, Meareg Amare, 2020). Improper use or insufficient withdrawal periods can lead to its residues in animal-derived food products like milk, meat, and eggs. The overuse of tetracycline in agriculture and medicine contributes to the development of antibiotic-resistant bacteria, a growing global health concern. (Jose Luis Martinez, 2009)

Monitoring tetracycline levels can help enforce regulations to curb overuse and misuse. Tetracycline residues can enter water systems through agricultural runoff and improperly treated waste, promoting the spread of resistance genes in the environment. Regulatory agencies, such as the FDA, EMA, and WHO, set maximum residue limits (MRLs) for antibiotics in food products to ensure consumer safety (Mohammad Oves, Mohammad Omaish Ansari, Mohammad Zain Khan, Mohammad Shahadat, Iqbal M.I. Ismail, 2020).

Quantification of TET concentrations in water and soil helps assess the extent of pollution from agricultural and industrial sources and its potential ecological impact. The determination of these analytes by classical chromatographic methods often requires long analysis time, significant costs

and qualified personnel. (Fatimah Alanazi, Rawan Almugbel, Hadir M Maher, Faten M Alodaib, Nourah Z Alzoman, 2023), (Ewelina Patyra, Krzysztof Kwiatek, 2016).

In the other side, electrochemical-based biosensors methods that convert a biological event to an electrochemical signal that can be measured, offer important advantages related with simplicity, low cost, sensitivity and selectivity. (Seyed Hamid Jalalian, Niloofar Karimabadi, Mohammad Ramezani, Khalil Abnous, Seyed Mohammad Taghdisi, 2018).

For this purpose, has been studied the possibility of developing an electrochemical sensor to quantify tetracycline. The sensor is based on carbon paste and modifiers, increasing selectivity, reducing detection limit and analysis time and all operating conditions are optimized. (Pavlína Landová, Milada Vávrová, 2017)

Carbon paste electrodes (CPEs) can be easily modified with various materials, such as nanoparticles, polymers, enzymes, or molecularly imprinted polymers (MIPs), to enhance selectivity and sensitivity. The electrode surface can be renewed by simply replacing or reshaping the paste, ensuring consistent performance and avoiding fouling issues (Alfredo J. T. Bosco, Eliana M. Alhadeff, Francisca das C. S. S. Mihos, Lídia Yokoyama, Victor M. Santos, Ninoska I. Bojorge Ramirez, 2015). They exhibit a broad potential range suitable for many redox reactions includes antibiotics. (Nagwa Abo El Maali, Azza M.M. Ali, Mahmoud Khodari, M.A. Ghandour, 1991)

2-Materials and methods

1.1 Electrochemical methods

Electrochemical methods such as Square Wave Voltammetry (SWV) and Differential Pulse Voltammetry (DPV) are widely used for the sensitive and selective determination of antibiotics (Louis Ramaley, Matthew S. Krause, 1969). SWV applies a square-wave potential superimposed on a staircase potential, the current response is measured as the difference between the forward and reverse pulses and this differential approach enhances sensitivity and reduces background noise. DPV applies a series of potential pulses superimposed on a linear sweep, the current is measured just before the end of each pulse to reduce the influence of capacitive currents and the difference

between currents at successive pulses is recorded to produce a voltammogram. (Valentin Mirceski, Sławomira Skrzypek, Leon Stojanov, 2018)

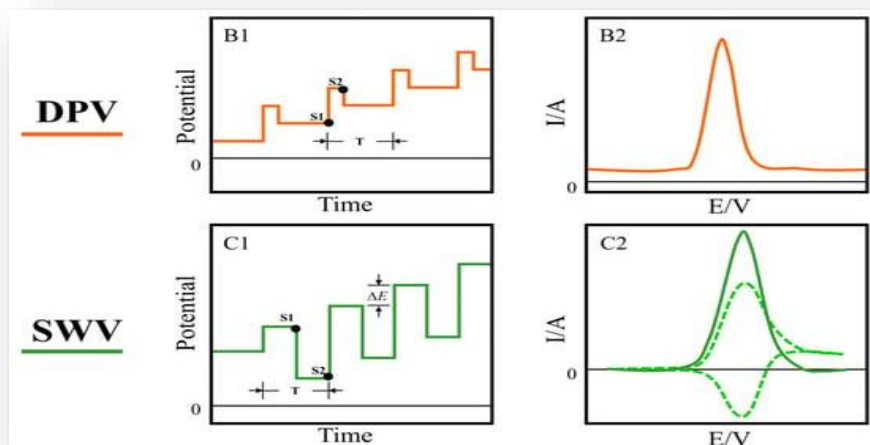


Figure 1 The potential waveform (B1) and voltammogram (B2) of differential pulse voltammetry, in the potential waveform, T is the waveform period, and S1 and S2 are the two current sampling points; the typical potential waveform (C1) of square wave voltammetry, ΔE is the potential increment, T is the potential period. The response current consists of forward (anodic current) and reverse (cathodic current) components (dashed line in (C2)), and their difference results in a net current (solid line in (C2)) (Jingjing Liu , Yifei Xu, Shikun Liu, , Shixin Yu, Zhirun Yu, Sze Shin Low, 2022)

All electrochemical measurement, in this study are performed with PalmSens 4 (PalmSens, De Indruk, The Netherlands), potentiostat–galvanostat, running three-electrode system, a platinum electrode was the counter, Ag/ AgCl/Cl⁻ (in KCL 3M) as the reference electrode and the carbon paste (CPE) as working electrodes.

1.2 Carbon paste electrodes

The unmodified carbon paste sensor is prepared by mechanically mixing graphite powder with particle size of 70-90 μm with paraffin in certain ratios, optimized before, in an agate mortar for about one hour and then stored in a refrigerator at 4°C for 24 hours. The carbon paste is packed into about 1 cm of the length of the syringe where a copper wire is placed to enables electrical contact.

1.3 Modified carbon paste electrodes

The modified sensor is prepared in similar way as the unmodified sensor by adding the modifier during homogenization of the carbon powder and paraffin. Than the composite is placed in a refrigerator (4°C) for 24 hours, then packaged in the body of sensor. The table 1 summarizes the ratio between each component used for different modified CPEs.

Table 1. The amounts of carbon powder, paraffin, and amount of modifiers used to prepare the different types of modified CPE

No.	Carbon powder (71-91 μm)	Binder	Type of modifier	Amount of Modifier (g)
1	1 g	Paraffin 300 μL	-	-
2	1 g	Paraffin 300 μL	Graphene oxide (GO)	0.1
3	1 g	Paraffin 300 μL	Reduce Graphene oxide (rGO)	0.1
4	1 g	Paraffin 300 μL	Zinc oxide (ZnO)	0.1
5	1 g	Paraffin 300 μL	ZnO	0.2
6	2 g	Paraffin 600 μL	GO and ZnO	0.1 and 0.1

7	1 g	Paraffin 300 μL	GO in which is doped ZnO (GO@ZnO)	0.1
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2.4. Reagents and modifiers.

All reagents were of analytical grade (Sigma and Merck, by Merck, KGaA Darmstadt, Germany). Synthetic graphite powder (90–71 μm particle size) was obtained from Alfa Aesar (99.9% Alfa Aesar, by Thermo Scientific Chemicals, MA, USA), and paraffin oil (Olio di Vaseline, by Humanitas, Milano, Italia) was supplied by Zeta Farmaceutici (Sandrigo VI, Italy). A TET freshly stock solution was prepared in distillate water and refrigerated at 4 °C.

The ZnO nanoparticles are prepared following the recommended procedure: in 1.43 g $\text{ZnSO}_4 \times 7\text{H}_2\text{O}$, add 5.23 g $\text{Na}_3\text{C}_6\text{H}_9\text{O}_9$ and 100mL H_2O while mixing (lightly). Then add 1 g NaOH and leave the mixture for 2 hours in stirring conditions. The mixture is transferred to a plastic tube and perform centrifugation (5 min, 10000 rpm, RTP). Wash the precipitate (3 times with distillate H_2O and leave it at 60°C for 12 hours. (Vinoda B. Patil, Davalasab Ilager, Suresh M. Tuwar, Kunal Mondal, Nagaraj P. Shetti , 2022)

GO was prepared using Hummer method slightly modified. 1.5 g of graphene, 1.5 g of sodium nitrate (NaNO_3) and 69 mL of concentrated sulfuric acid (H_2SO_4 cc) was added in a beaker and the mixture was continuously stirred until a homogeneous solution was achieved. Then, the beaker was placed in a container filled with ice and continuously stirred for 15 minutes. 9 g of potassium permanganate (KMnO_4) was slowly added to the mixture and the beaker was placed in continuous magnetic stirring for 1 hour at room temperature. Afterwards, again it was transferred to a container with ice and 100 mL of distilled water was slowly added to the solution. Then, heated in a furnace at a temperature exceeding 90°C for 1 hour. After heating, 300 mL of distilled water were added and 10 mL of H_2O_2 30%. The solution was placed in an ultrasonic bath for 30 minutes at a temperature of 25°C.

Then 0.1 g GO, 0.1 g ZnO and 15 mL distilled water mixed for 24 h in RTP, then for 4 h the mixture is placed in an ultrasound bath and for 5 min in centrifugation at 5000 rpm, then dried in a thermostat for 4 h at 50° C.

Carbon powder is homogenized in a mortar with GO@ZnO and paraffin and finally packaged in a syringe, ready to measurement. (Maxim K. Rabchinskii, Maksim V. Gudkov, Dina Yu. Stolyarova, 2021).

2. Results and discussion

2.1 Optimization of the modifier

Modified CPE shown in Tab 1 are tested in an electrochemical cell of 15 mL of 0.1 M acetate buffer with pH 5. All measurements are performed using the SWV technique under the following conditions $t_{eq} = 10$ s, $E_{beg} = 0$ V, $E_{end} = 1.5$ V, $E_{step} = 5$ mV, Amplitude = 50 mV, frequency = 10 Hz. The signals obtained due to application of standard additions of TET solution are used to construct the calibration graphs. Tab 3 shows the linear equations that represent the behaviour of each electrochemical sensor. Obviously CPE modified with ZnO shows the highest sensitivity.

Table 3. TET calibration curve equation for different types of modified CPE

CPE	$y = 97.611x + 18.438$
CPE GO	$y = 31.685x + 55.158$
CPE rGO	$y = 42.737x + 6.3127$
CPE ZnO	$y = 151.58x + 33.672$
CPE ZnO (X2)	$y = 224.05x + 39.826$
CPE GO, ZnO	$y = 142.24x + 37.19$
CPE GO@ZnO	$y = 182.88x + 44.629$

2.2 Modifier characterisation

A morphological characterization of the surface of the ZnO modified CPE was performed using SEM and EDX techniques (Fig.2, Fig. 3)

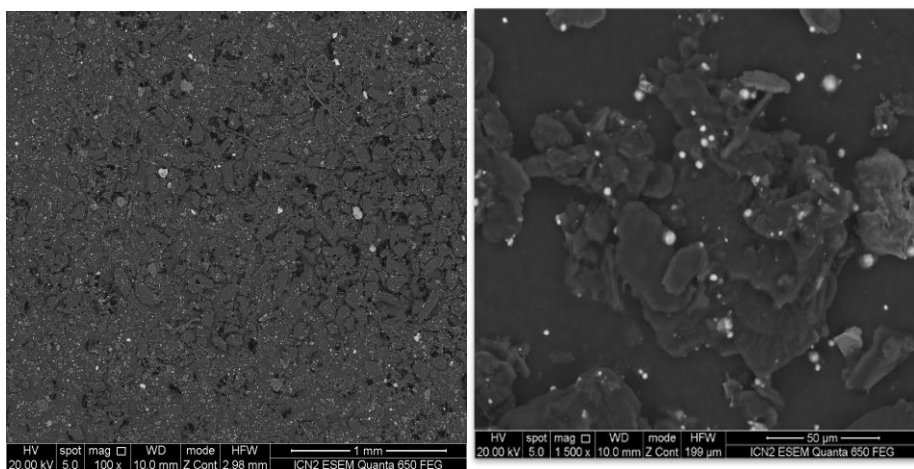


Figure 2 SEM (Different magnification) for the CPE modified with ZnO

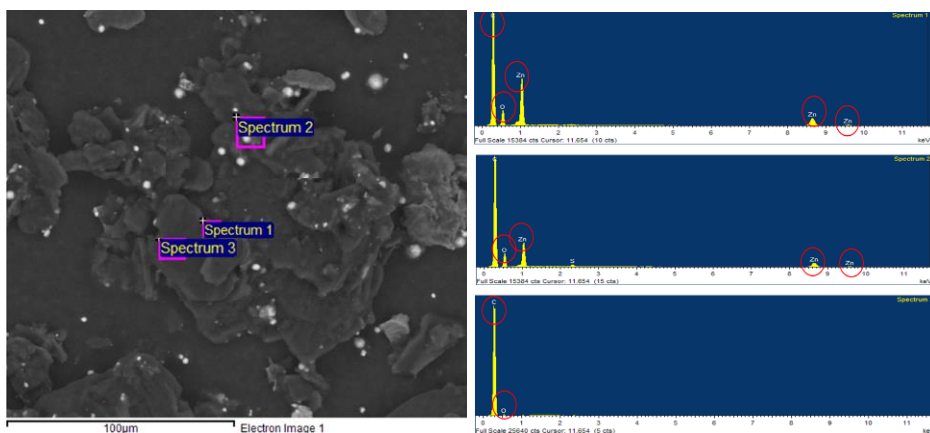


Figure 3 EDX spectrum of the CPE modified with ZnO

It is noted that the modifiers used are well distributed in the carbon paste and the size of the particles corresponds to their nanometre order.

2.3 Optimization of the buffer and pH

Optimisation of pH and the indifferent electrolyte is done using the CPE modified with 20% synthesized ZnO. Measurements were performed using SWV technique following the above mentioned conditions in 15 mL

indifferent electrolyte. The peaks current obtained in the presence of TET 0.5mM are summarized in the Table 4

Table. 4 The peak current (μA) displayed for different types of buffer, in the presence of 0.5 mM TET. SWV technique is used with these conditions $t_{\text{eq}}=10$ s, $E_{\text{beg}} = 0$ V, $E_{\text{end}} = 1.5$ V, $E_{\text{step}} = 5$ mV, Amplitude = 50 mV, frequency = 10 Hz

Types of buffer	I of peak (μA)
a. Acetate buffer 0.1 M, pH 5	136.6
b. Acetate buffer 0.2 M, pH 5	158.9
c. Acetate buffer 1 M, pH 5	150.0
d. phosphate buffer 0.1 M, pH 7.5	140.5
e. PBS buffer 0.1 M, pH 7.5	131.1
f. PBS buffer 0.1 M, pH 8	125.4
g. PBS buffer 0.1 M, pH 6.5	113.9
h. TRIS-HCl 20 mM, NaCl 50 mM, pH 7.5	30.8
i. TRIS-HCl 20 mM, NaCl 50 mM, 10 mM MgCl_2 , pH 7.5	104.1
j. TRIS-HCl 20 mM, NaCl 100 mM, MgCl_2 2 mM, KCl 5 mM, CaCl_2 1 mM, pH 7.5	46.2

As it is shown, the peak current intensity is better displayed by acetate buffer 0.2 M, pH 5. Because of pH is generally important factor in the progress of a redox reaction further optimization are performed. The dependence of peak current from the pH of acetate buffer 0.2 M is studied. In Fig 4 are shown the SWVs recorded in different pH of acetate buffer in the presence of the same concentration of TET (0.5 mM).

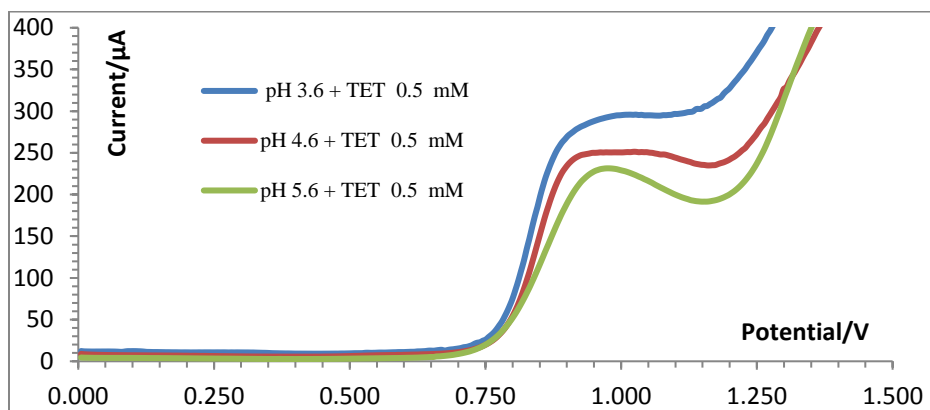


Figure 4 Voltammograms of SWV technique (t_{eq} 10 s, E_{beg} 0 V, E_{end} 1.5 V, E_{step} 5 mV, Amplitude 50 mV, frequency 10 Hz), in 15 mL of acetate buffer 0.2 M with different pH and addition of 0.5 mM TET.

From the shape of the voltammograms the pH 5.6 is more suitable for identification of TET measurements compared to the others cases.

2.4 Optimization of the working condition for SWV and DPV technique

Working parameters of SWV for detection of TET are optimized using the electrolyte cell 0.2 M acetate buffer with pH 5.6 and 0.5 mM of TET varying the E_{step} (1mV- 10mV), Amplitude (10 mV- 100 mV), Frequency (1 Hz - 50 Hz), T_{eq} (60 s - 0 s). Based on the shape of the peak current, the best parameters resulted: T_{eq} = 60 s, E_{beg} = 0.5 V, E_{end} = 1.5 V, E_{step} = 5 mV, Amplitude = 50 mV and Frequency = 100 Hz.

2.5 Analytical Performance of CPE/ZnO(x2) for TET Determination

Analytical performance of the CPE/ZnO(x2) for TET determination is studied using SWV under the optimized parameters and three electrode system: CPE/ZnO(x2) (working), Ag/ AgCl/ Cl⁻ (reference electrode) and Pt (counter electrode). The addition of standard solution of TET are applied in 15 mL of 0.2 M acetate buffer and pH 5.6. The voltammograms are shown in fig 4 and the dependence of peak current to TET concentration in Fig 5.

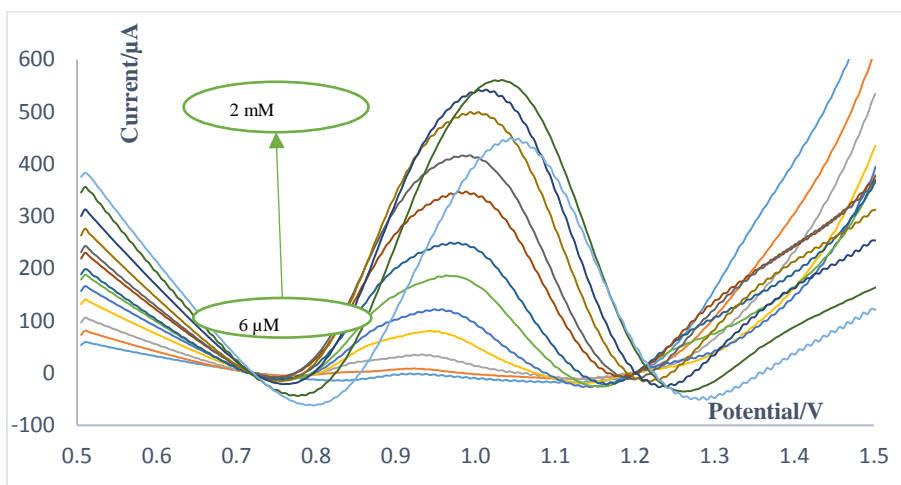


Figure 4 Voltammograms for SWV measurements with $t_{eq} = 60$ s, $E_{beg} = 0.5$ V, $E_{end} = 1.5$ V, $E_{step} = 5$ mV, Amplitude = 50 mV, frequency = 100 Hz of the ZnO 20 % carbon paste electrode. TET concentrations varied 6 μ M–2 mM in acetate buffer at pH 5.6

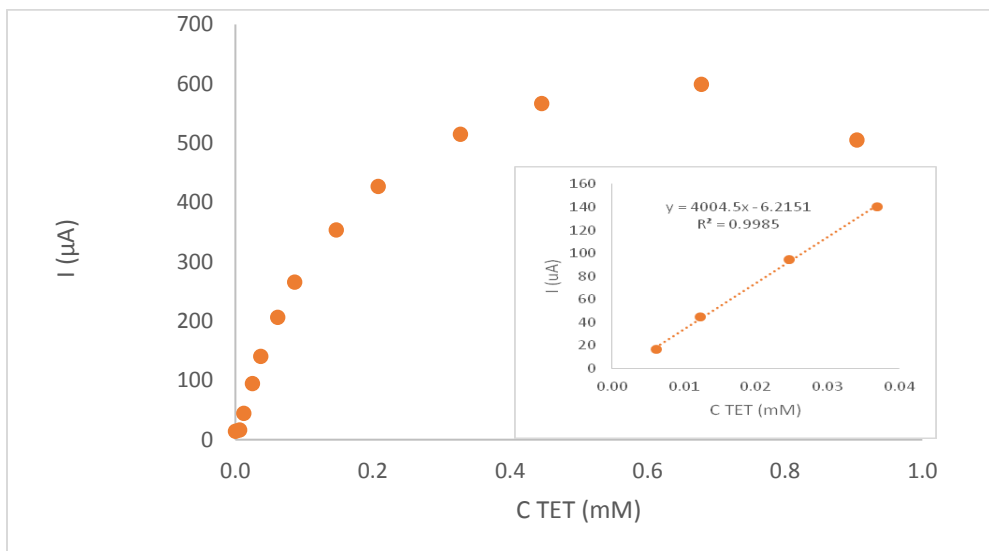


Figure 5 a) calibration curve and b) linear range derived from SWVs ($t_{eq} = 60$ s, $E_{beg} = 0.5$ V, $E_{end} = 1.5$ V, $E_{step} = 5$ mV, Amplitude = 50 mV,

frequency = 100 Hz), using CPE modified with 20% ZnO, in acetate buffer pH 5.6. Concentration of TET 6 μ M -2 mM

Referring to three replicates in linear range of calibration graph the analytical performance parameters are calculated and are shown in Table 5.

Table 5 Analytical performance parameters for measurements with the SWV technique under optimized conditions, in 0.2 M acetate buffer, pH 5.6, with the CPE/ZnO(x2) electrode.

Exp	Sensi. μ A/ppm	Correla. coeffi.	Linear range ppm	LOD ppm	RSD (%)
1	7.9	0.9962	3-18	4.4	9.4
2	8.3	0.9985	3-18	4.2	
3	7.7	0.9971	3-18	5.2	

3.6 Study of interferences in the determination of TET

Possible interferences that could affect the quantification of TET using the developed sensor under the optimal chemical and technical conditions are studied. SWVs are recorded in the presence of other antibiotics such as Ampicillin, Azithromycin and Penicillin. In the optimized electrochemical cell and in the presence of 10 μ M interferent (AZI/AMPIC/PEN), are recorded SWVs after each TET additions. In Fig. 7 are shown the calibration graphs in the presence and absence of interferences. It is shown that the three antibiotics affect the signal of TET consequently the sensitivity is decreased. The interference of AZI resulted higher due to the fact that both of these antibiotics (TET and AZI) have the oxidation potentials close to each other.

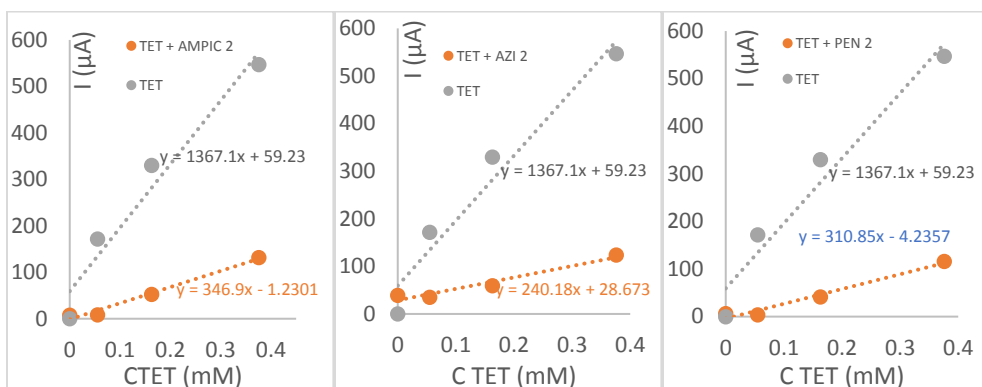


Figure 7 Calibration graphs of TET using CPE/ZnO in the presence and absence of interferent (AMPIC, AZI, PEN)

3.7 Determination of TET in synthetic sample

The standard addition method is applied to test the developed sensor using a synthetic sample containing TET. The calibration graph of standard addition methods in optimised parameters is shown in fig. 8.

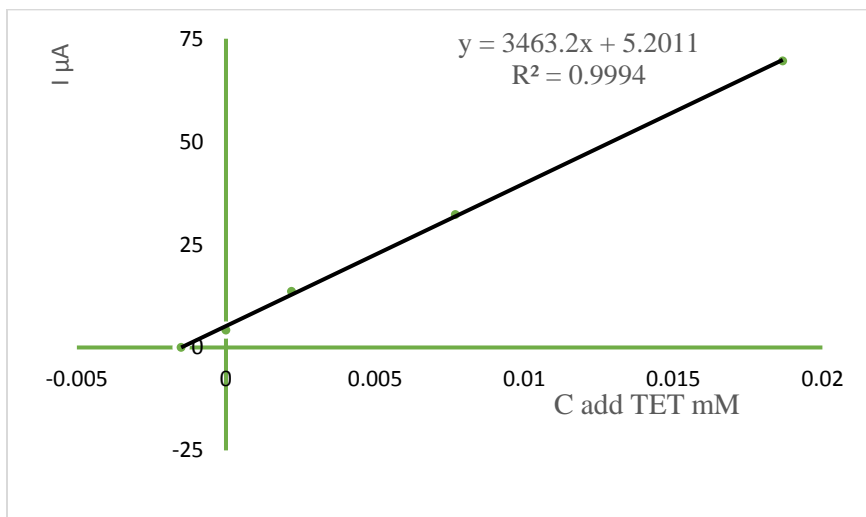


Figure 8 Standard addition method for a synthetic sample of TET measure with SWV ($T_{eq} = 600$ s, $E_{beg} = 0.5$ V, $E_{end} = 1.5$ V, $E_{step} = 5$ mV, Amplitude = 50 mV and frequency = 100 Hz) using CPE/ZnO

Based on the calculations performed, the concentration of TET in the synthetic sample containing 5 μM TET resulted to be approximately 4.5 μM corresponding to 80% accuracy of the result.

Conclusions

In this paper was developed a new sensitive and selective electrochemical sensor for antibiotic (TET) determination based in modification of CPE using ZnO nanoparticles. The modifier was successfully synthesized in the lab which was confirmed by characterization techniques (SEM and EDX). The CPE-ZnO sensor displays a good sensitivity (8 $\mu\text{A/ppm}$), in a linear working range up to 1mM, with correlation coefficient $R=0.9972$ and detection limit of 4.5 ppm.

The proposed sensor was tested in the determination of TET in synthetic samples. Other antibiotics interfere the TET determination, which can be avoided using the standard addition method. In conclusion, the proposed modified CPE shows great promise for the determination of TET-contaminated samples.

Acknowledgements

The authors gratefully acknowledged support from SUSNANO project 101059266 “Twinning to boost the scientific and innovation capacity of the Universiteti i Tiranës to develop sustainable nanosensors for water pollution detection”. The authors gratefully acknowledged the assistance provided by the Institut Català de Nanociència i Nanotecnologia (ICN2), Barcelona, Spain for performing the morphological characterizations SEM and EDXS measurements. The authors would like to thank also NASRI for great support in the frame of NASRI-TUBITAK bilateral project.

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