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Development of unmodified and CTAB modified carbon paste electrodes (CPE) for direct electrochemical analysis of nitrites in aquarium water and rat blood

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Abstract

The present paper deals with the development of a new and original hexadecyltrimethylammonium bromide (CTAB) modified carbon paste electrode (CPE) dedicated for the direct analysis of nitrites in aquaculture and rat blood. The surface morphologies and contact angles of unmodified and modified CPEs were performed using Scanning Electron Microscopy (SEM), Optical Microscopy (OM) and sessile drop techniques, respectively. Cyclic Voltammetry (CV) was used to study the electrochemical behaviour of nitrites, while Differential Pulse Voltammetry (DPV) and Linear Sweep Voltammetry (LSV) techniques were used to investigate their quantitative analysis. DPV operating parameters were optimized and the optimum values were: pulse height 0.4 V, scan increment 0.015 V, pulse width 0.01 s and step time 0.5 s. The repeatability and reproducibility of the CPE was studied, and the effect of some interfering ions was evaluated to highlight the selectivity of the electrode. The modified CTAB/CPE showed a good stability and proved to be more sensitive, leading to a Limit of Detection (LOD) of 0.034 mg L⁻¹ and a Limit of Quantification (LQ) of 0.152 mg L⁻¹. The elaborated sensors were applied with success for nitrites analysis in aquaria with CPE and in rat blood with CTAB/CPE, preparing human blood measurements in near future work.

Keywords: CPE; CTAB; Nitrites; Aquaculture; rat blood

1- Introduction

The nitrite ion is one of the active intermediate species in the nitrogen cycle and a useful indicator of equilibrium state of the oxidative and reductive pathways of the nitrogen cycle. It is also an intermediate species in soil and water ecosystems as by-product in the reduction of nitrate. Because of the extensive use of nitrites as an additive or preservative in food industries, fertilizer and as corrosion inhibitors, it is frequently found in natural water, soil and food [1-3] and subsequently in human beings and animals [4]. The excessive presence of nitrites in different ecosystems may pose a real risk and hazard to human and animal health. Nitrites cause the malfunctioning of the haemoglobin whose role is to transport oxygen in blood to other parts of the body. A higher level of nitrites may lead to methemoglobinemia commonly known as “blue baby syndrome” in babies [5,6]. The drastic levels of nitrites can cause brain damage and eventually results to death due to lack of oxygen [7]. As typical example, in aquaria, ammonia is produced as fish wastes, uneaten food and decaying plant matter which is biologically converted into nitrites that the highest levels kill fish and invertebrates. The toxic doses of nitrites in aquaria vary and depend on the fish species, but the value of 0.2 mg L^{-1} is known to be the acceptable level in surface water, 20 mg L^{-1} in seawater but only 0.1 mg L^{-1} for potable water. In aquaria, nitrite levels above 1 mg L^{-1} may cause stress in fishes and lead to their death when levels are greater than 4 mg L^{-1} [8]. For this reason, the nitrites levels in aquaria/environment need to be continuously monitored.

In the same line, in animal bodies, nitrates are firstly converted into nitrites by bacteria present in the saliva, stomach, and intestines. Then, the formed nitrites will primarily react by oxidizing the iron component of red blood cells (haemoglobin) to methemoglobin, rendering them unable to carry oxygen. This may lead to the ischemia-reperfusion phenomenon, which occurs when a tissue or an organ is no longer sufficiently irrigated with arterial blood. Thus, a decrease in blood supply

will lead to a drop in the partial oxygen pressure of the organ in question, also called hypoxia. Consequently, the cellular energy metabolism is altered and the mitochondria is no longer able to ensure the oxidative phosphorylation necessary for the production of ATP: aerobic glycolysis is then stopped. Anaerobic metabolism will take over to produce energy but it will be of low yield and will thus lead to the accumulation of free radicals (reactive oxygen species, i.e. NO, peroxy-nitrites) and lactates [9,10]. The lethal dose of nitrite has been reported to be 300 mg kg⁻¹ per body weight [11]. Thus, due to the toxic effects of nitrites for human and fish health, the levels of this substance need to be monitored regularly and kept below 0.5 mg L⁻¹ to avoid possible toxicity [12]. Hence, the development of effective and sensitive methods for nitrite detection is essential for human health and environmental monitoring. Numerous analytical methods have been used for the detection and quantification of nitrites, such as spectrophotometry [13,14], chemiluminescence [15], chromatography [16], micro-spectrophotometry with liquid-phase microextraction [17], capillary isotachopheresis [18], microchip electrophoresis [19], raman spectroscopy [20], gas chromatography-mass spectrometry [21] and electrochemical methods [22, 23]. All the mentioned methods are helpful, but most are relatively time-consuming operations, complicated experimental conditions, using toxic reagents and expensive equipment, which hinder their further applications. Electrochemical methods have shown to be preferable over others methods for the determination of nitrites due to their fast response, low-cost and simplicity to handle, with the possibility of portable measurements. Moreover, electrochemical experiments take place on the surfaces of the electrode that have the possibility to be modified by active molecules with the aim to increase their sensitivity leading to the determination of trace level of nitrites.

The present work proposes the development of an unmodified and a CTAB modified carbon paste electrodes for nitrites analysis in a domestic aquarium water and in Wistar rat blood, respectively.

2- Material and Methods

2.1 Reagents and solutions

NO_2^- solution at 10 g L^{-1} was prepared using NaNO_2 , purchased from Merck company (Lot # C868979). Hexadecyltrimethylammonium bromide (denoted CTAB) salt was purchased from Merck company (Lot # SLCK0940). HAS (Human Serum Albumin) preparation at 20 g L^{-1} was obtained using HAS VIALEBEX 200 mg mL^{-1} (Lot # 21L01195), purchased from LFB Biomedicaments (France). Phosphate buffer solution (PBS, pH = 7.4, ionic strength 0.1 M) was obtained from the following salts (pure grade, for analysis): $\text{H}_2\text{PO}_4\text{Na}$ and HPO_4Na_2 . Both salts were purchased from Merck company. Ultrapure water was elaborated from Elga epure system from Elga Lab water company. The ultrapure water used showed a pH of 6.5 and DOC < 0.1 ppm.

2.2 Microscopies characterizations of CPE materials

The optical analysis was conducted on the CPE surfaces with a VHX-7000 digital microscope from Keyence SAS company (France). The mean roughness Ra which is the mean value of the height of points of the surface relative to the center plane within the defined area, was calculated following the given [Equation 1](#):

$$Ra = \frac{1}{L_x L_y} \int_0^{L_y} \int_0^{L_x} |z(x, y)| \cdot dx \cdot dy \quad (1)$$

where $z(x, y)$ is the height relative to the center plane. L_x and L_y represent the dimensions of the analyzed surface.

The CPE surfaces were also characterized by scanning electron microscopy with a field emission gun (FEG-SEM) using an LEO 1530 apparatus at a 3 keV acceleration voltage, allowing both

minimization of irradiation damages. Observations were conducted until $\times 5,000$ magnification. To increase the quality of the images, it was necessary to cover the analyzed surfaces with an ultra-thin layer of Pt (4 nm thickness) deposited by vacuum evaporation with BAL-TEC MED 020 (Balzers Liechtenstein) apparatus.

2.3 Preparation of the working electrodes

In the present study, unmodified and modified CPE were prepared and used for the electroanalysis of nitrites in a domestic aquarium and rat blood by following the methodology described by Mbokou *et al.*, 2019 [24]. Thus, for the unmodified CPE, 70 mg of graphite powder (analytical grade, ultra F, < 325 mesh, from Alfa) and 30 mg of silicon oil were thoroughly hand mixed in a mortar. The obtained composite mixture was inserted into the cylindrical hole of a teflon tube equipped with a copper wire, serving as electrical contact with the rest of the circuit. The surface exposed to the solution was polished on a weighing paper to give a smooth aspect before use. CTAB modified carbon paste electrodes (CTAB/CPE) was prepared similarly as the bare CPE but by mixing 65 mg of graphite powder, 30 mg of silicon oil and 20 μL of a CTAB solution at 0,091 g L^{-1} in water (under the critical micelle concentration). When not in use, both elaborated CPEs were kept at room temperature.

Electrochemical measurements were performed in a conventional three-compartment cell using a PC-controlled PG581 Uniscan instruments company. The working electrode (CPE, with 3 mm of diameter). A Platinum wire served as the auxiliary electrode while a saturated calomel saturated electrode was used as stable reference electrode and denoted SCE. The pH values of the solutions were determined with a WTW instrument (pH330i) pH meter. All experiments were performed at room temperature.

2.4 Measurements of contact angles

The sessile drop technique was used for contact angle measurements. Thus, a liquid droplet is placed on a flat homogeneous surface and the contact angle of the droplet with the surface is measured with a KRUSS DS10 contact angle metre. A droplet of MilliQ water solution (pH 6.5) with a volume of 100 nL was deposited on the surface using a micro-syringe and a camera measured the angles during the first second after water droplet deposition. Reported values are the averages of the contact angles (right and left) of three droplets.

2.5 Aquarium water and rat blood samples preparation

Water samples and fish species: Two water samples of 100 mL were collected from an aquarium containing nine *Symphysodon aequifasciatus* (discus) hybrid in 0.5 m³ of tap water at a temperature thermostated at 28°C. Nitrite ions were measured in both samples without any pre-treatment with the unmodified CPE using the standard method of known dose additions.

Blood samples and rat species: Two blood samples of 2 mL were deducted from one Wistar rat (*Rattus norvegicus*). Both samples were firstly pre-treated and then analysed by DPV technique on CTAB/CPE using the standard method of known dose additions. The pre-treatment was carried out in Tubes BD Vacutainer® plus PET sterile with BD Hemogard 5 mL under a vacuum, 2 mL 13 x 75 mm heparin-lithium cap green (Ref. 368494, BD Plymouth, UK).

3- Results and Discussion

3.1 Scanning electron microscopy (SEM) and optical microscopy (OM) characterizations

SEM observations realized at a magnification of x19 show a CPE disc diameter of 3 mm for the two tested electrodes, modified and unmodified, as seen in [Figure 1](#).

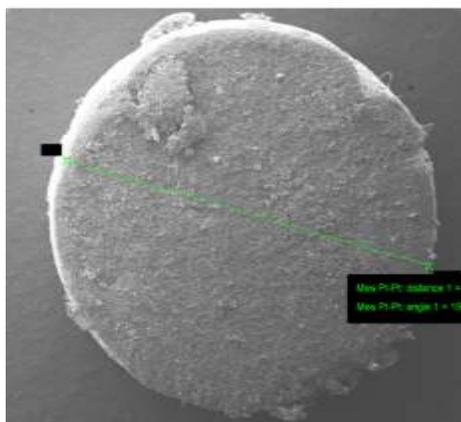


Figure 1: SEM image of the unmodified carbon paste disc

Both unmodified and CTAB modified CPEs have the same geometrical surfaces that were calculated to be 0.071 cm^2 .

The SEM 3D images of the bare CPE and the modified CTAB/CPE are presented in [Figure 2](#). No significant difference was observed on the surfaces of both tested electrodes.

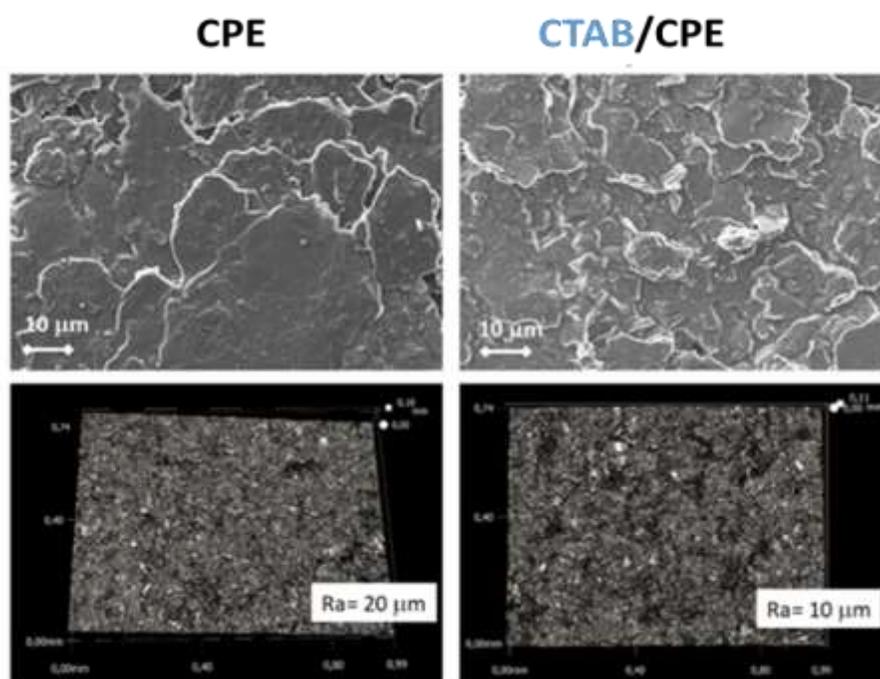


Figure 2: Unmodified and modified CTAB/CPE observed by SEM under a magnification of x5,000 and with OM under 3D analysis

In fact, SEM observations show very similar morphology in the presence and absence of the surface-active agent CTAB. The OM observation aided in the determination of the average roughness (Ra), which was estimated to be 20 μm and 10 μm for the CPE and CTAB/CPE, respectively. The halving observed for Ra values indicates the presence of CTAB in the carbon paste and then also proves the insertion of CTAB in the carbon paste.

3.2. Contact angle measurements

The hydrophilic character of unmodified and CTAB modified CPE was evaluated using sessile drop contact measurements. [Figure 3](#) presents the obtained results.

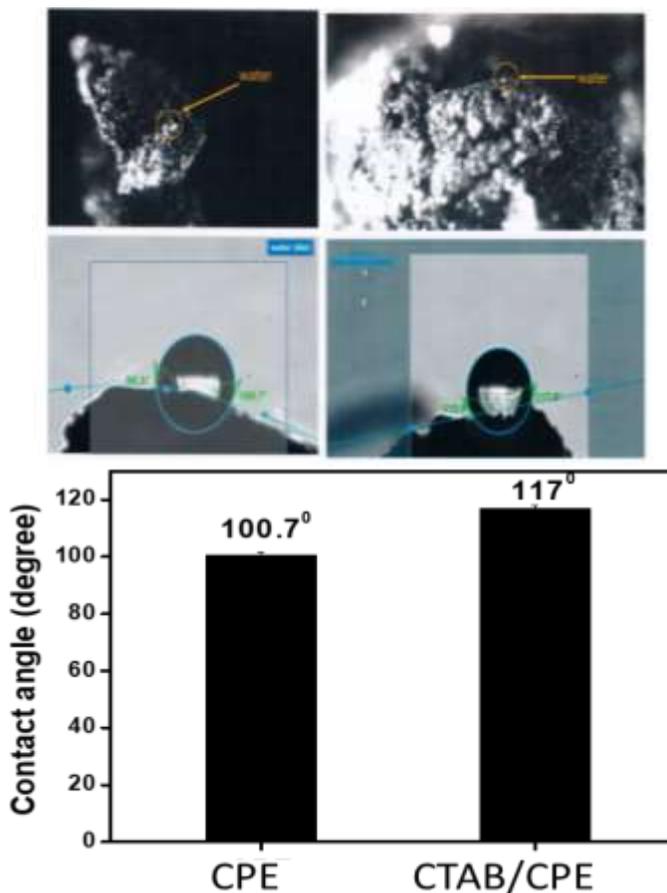


Figure 3: Contact angle measurement results occurred using the sessile drop technique applied to unmodified CPE and modified CTAB/CPE

From [figure 3](#), the contact angle measurements realized on both CPEs show values of $100.7^\circ \pm 1^\circ$ and $117^\circ \pm 1^\circ$ for unmodified CPE and CTAB/CPE, respectively. The obtained difference of 17° indicates the chemical change between the two tested electrodes, due to the presence of the surface-active agent CTAB in the carbon paste mixture. The significant increase observed in degree (17°) after electrode modification clearly traduces the hydrophobic character of the CTAB molecule.

We know that the effect of average roughness on contact angle measurements occurred for roughness higher than 100 nm, but the obtained results are in the range of 10-20 μm . As a consequence, we can't consider that the presence of CTAB may slightly influence the hydrophobicity increment observed vs roughness, but we can conclude that the presence of CTAB in the carbon paste is well demonstrated. It shows additional results with previous OM observations in [Figure 2](#).

3.3. Determination of Geometric and Real Surfaces for both CPEs developed

The geometric and active surfaces of both unmodified and CTAB modified CPE were evaluated. The geometric surfaces were calculated using the formula $S_g = \pi r^2$, where $\pi = 3.14$ and $r = 15 \text{ mm}$ is the geometric radius (r) of the electrodes, estimated from the SEM image of CPEs. By contrast, the active surface areas of both unmodified and CTAB/CPE were evaluated from the current intensity in 0.1 M PBS containing 5 mM ferricyanide on each electrode using cyclic voltammetry as presented in [figure 4](#).

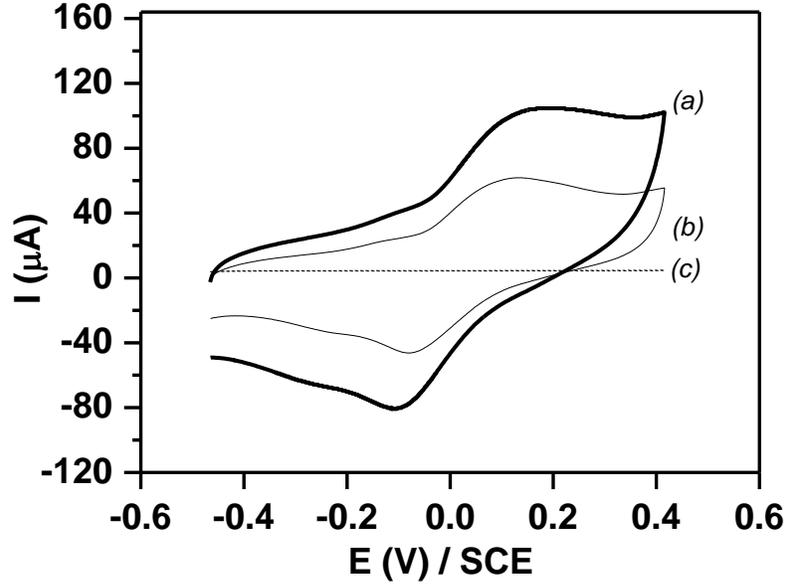


Figure 4: Cyclic voltammograms recorded in 0.1 M PBS containing 5 mM ferricyanide on (a) CPE, (b) CTAB/CPE. Curve (c) corresponds to the CV behavior in blank supporting electrolyte (Potential scan rate: 100 mV s⁻¹)

As seen in [figure 4](#), the peak intensity (I_p) of ferricyanide at each given electrode was used for the determination of real surface area (A) of that electrode on the basis of well-known Randles-Sevcik [Equation \(2\)](#):

$$I_p = k \cdot n^{3/2} \cdot A \cdot D^{1/2} \cdot C \cdot V^{1/2} \quad (2)$$

where $k = 2.69 \times 10^5$, n is the number of moles of electrons transferred per mole of electroactive species, A (cm²) is the area of the electrode, D (cm² s⁻¹) is the diffusion coefficient of the electroactive species, C (mol L⁻¹) is the bulk concentration of the electroactive species, and V (V s⁻¹) is the potential scan rate [25]. The obtained results are presented in [Table 1](#).

Table 1: Geometric and real surface area of unmodified CPE and modified CTAB/CPE

Electrodes	Geometric surface area (S_g) (cm ²)	Real surface area (A) (cm ²)
CPE	0.071 ± 0.005	0.129 ± 0.005
CTAB/CPE	0.071 ± 0.005	0.096 ± 0.005

From [table 1](#), the geometrical areas S_g of the both tested electrodes were found to be 0.071 cm^2 . The real surface area (A) of the unmodified CPE was found to be 0.129 cm^2 and that of the CTAB/CPE was 0.096 cm^2 . The presence of the CTAB in the carbon paste decrease by 20% the surface area completing the observations of the average roughness decreased previously. Furthermore, these results come to prove again the presence of CTAB in the modified CPE.

3.4. Electrochemical behavior of nitrites at different electrodes

The electrochemical behavior of 10 mg L^{-1} nitrites on the elaborated electrodes was studied by cyclic voltammetry in 0.1 M PBS at pH of 7.4 as presented in [figure 5](#).

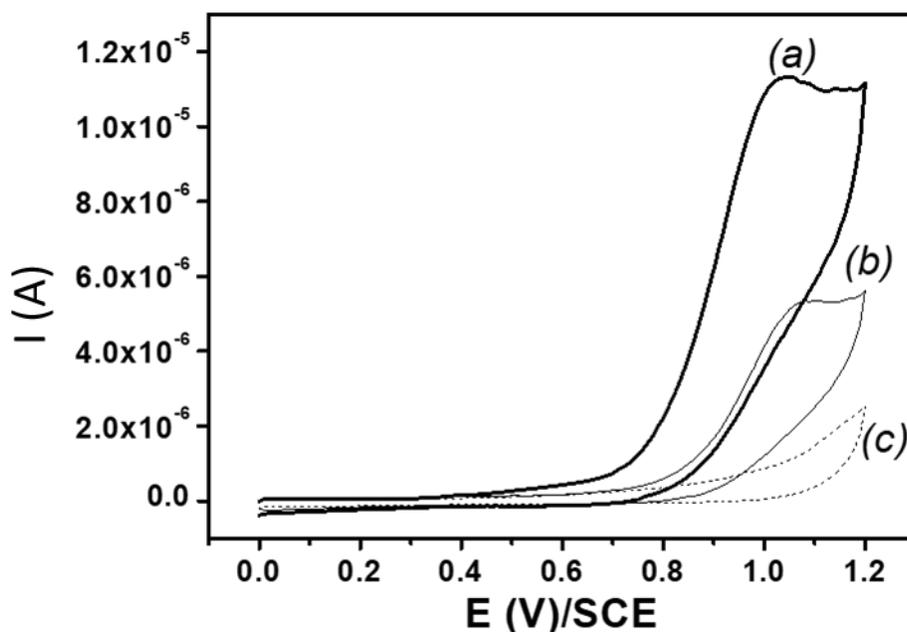
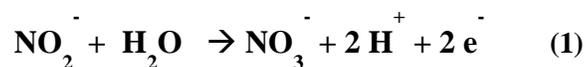


Figure 5: Cyclic voltammograms recorded in 0.1 M PBS ($\text{pH} = 7.4$) containing 10 mg L^{-1} of nitrites on (a) CPE/CTAB, bare CPE (b). Curve (c) corresponds to the CV behavior in blank solution, at a potential scan rate of 100 mV s^{-1}

As seen in [Figure 5](#), all the obtained voltammograms are irreversible for both tested CPEs. The voltammogram obtained on the CTAB/CPE is more pronounced than that obtained on the bare CPE in terms of peak intensity and sharpness. The peak current intensity is found to be $11 \mu\text{A}$ ([Figure 5\(a\)](#)) with CTAB/CPE while the unmodified CPE shows a peak current intensity of $6 \mu\text{A}$

(Figure 5(b)). Also, the lower peak potential is observed at 1.02 V/SCE with the CTAB/CPE, while with the unmodified CPE the peak potential is at 1.06 V/SCE.

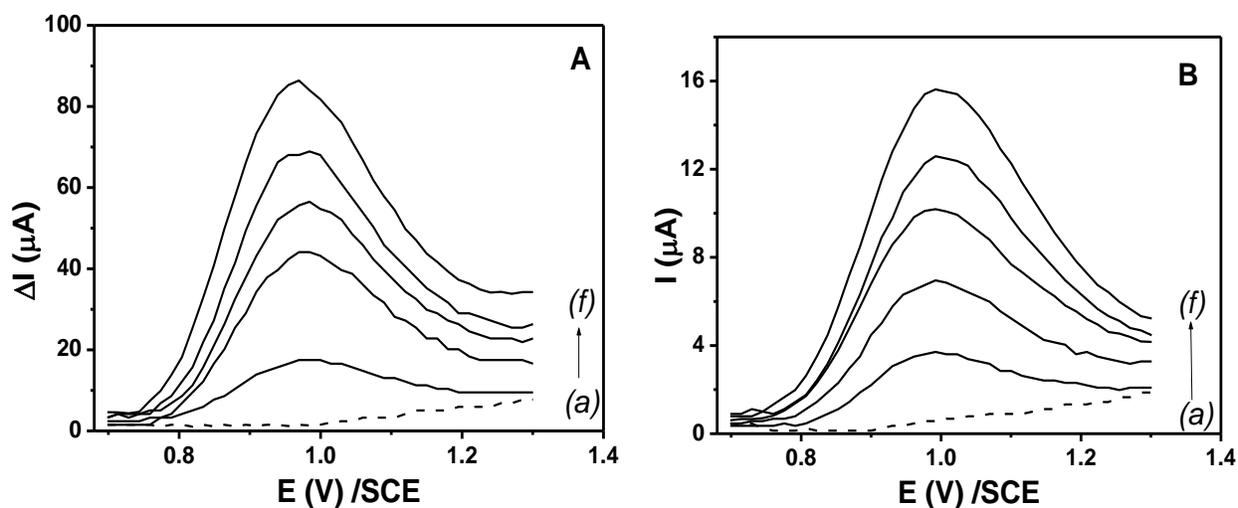
The principle of the developed CPEs is based on the direct oxidation of nitrites to nitrates as illustrated by the following half-redox reaction (1):



3.5. Comparison between differential pulse voltammetry (DPV) and linear Sweep voltammetry (LSV) techniques

Two electrochemical techniques, LSV and DPV were compared towards the direct oxidation of nitrites at a potential of 1 V/SCE on unmodified CPE. Thus, two calibration curves were plotted in the range of nitrite concentrations from 0 to 10 mg L⁻¹ on the CPE in LSV and DPV respectively.

Figure 6 presents the obtained results.



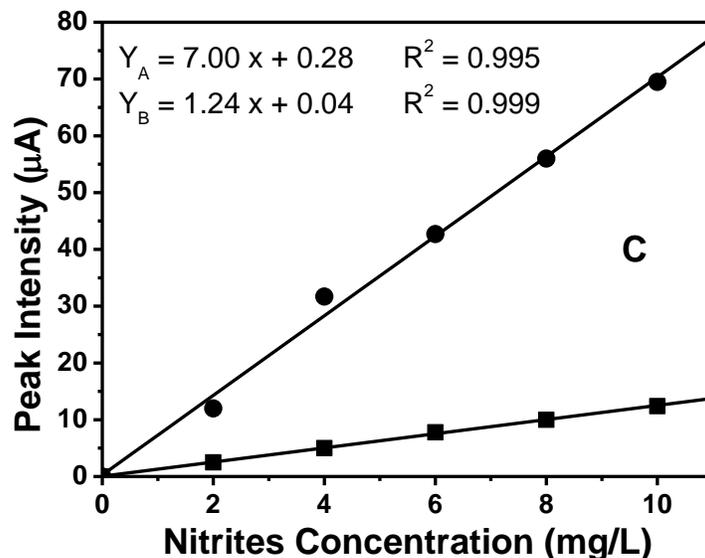


Figure 6: (A)- DPV and (B)-LSV of CPE in presence of (a-f): 0, 2, 4, 6, 8 and 10 mg L⁻¹ nitrites in 0.1 M PBS (pH 7.4). (C) shows peak current versus nitrite concentration.

As observed in the [Figure 6C](#) both obtained slopes are different for the two voltametric techniques. DPV technique shows a better slope compared to the LSV technique. This clearly indicates that the DPV technique is more sensitive than LSV, as expected. The limits of detection calculated using the formula $3S_b/m$ where S_b represents the standard deviation and m the slope were found to be 0.904 mg L⁻¹ and 0.367 mg L⁻¹ for LSV and DPV, respectively. From the results obtained in this section, DPV technique was chosen for the rest of experiments. To improve the DPV sensitivity, some instrumental parameters need to be optimized. Thus, the next section will be focused to that aspect.

3.6. Optimization of the DPV parameters

In order to find optimum conditions for which better electrochemical responses may be obtained, DPV instrumental parameters were varied by recording oxidation currents in a solution of 0.2 mg L⁻¹ nitrites on the unmodified CPE. Each of the following parameter: pulse height, scan increment, pulse width and step time was varied by maintaining others fixed. The obtained results are presented in [Figure 7](#).

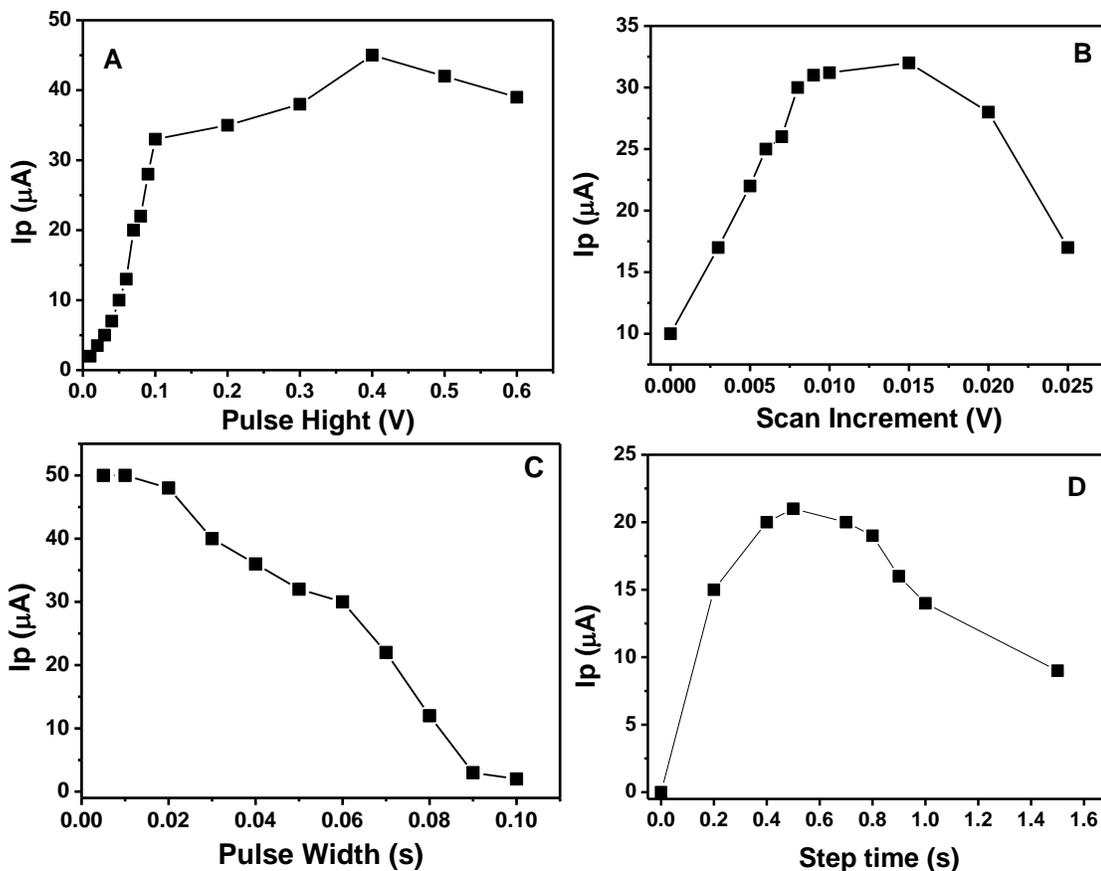


Figure 7: Evolution of peak intensity vs DPV parameters: (A) pulse height, (B) scan increment, (C) pulse width and (D) step time (experiments done on CPE in 0.2 mg L^{-1} nitrites in PBS)

Figure 7 shows the evolution of the oxidation peak current of nitrites as a function of each DPV parameter: pulse height, scan increment, pulse width and step time. Thus, the optimal values for each parameter tested individually (Figure 7) were 0.4 V pulse height, 0.015 V scan increment, 0.01 s pulse width and 0.5 s step time. These obtained optimum parameters were kept for the rest of experiments. Thus, a new calibration curve was plotted in the range of nitrite concentrations from 0 to 10 mg L^{-1} on the unmodified CPE. Figure 8 presents the obtained results.

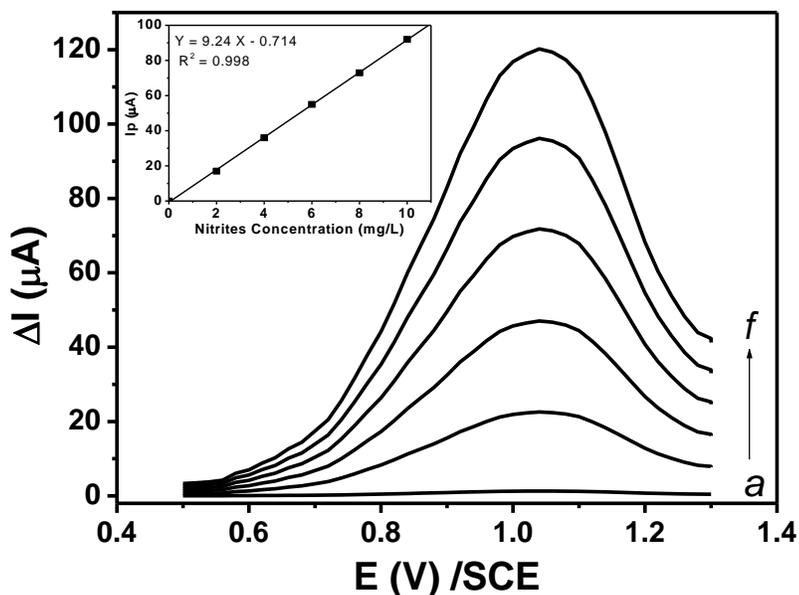


Figure 8: DPV of CPE in presence of (a-f): 0, 2, 4, 6, 8 and 10 mg L⁻¹ nitrites in 0.1 M PBS (pH 7.4). Insert shows peak current versus nitrites concentration

From [Figure 8](#), the sensitivity of the unmodified CPE improved significantly as a consequence of DPV parameter optimisation. The obtained slope of 9.24 μA mg⁻¹ L is better in comparison to 7.00 μA mg⁻¹ L obtained before the optimization of parameters. The gain of 20% on the slope value led to reach a detection limit and the quantification limit of 0.189 mg L⁻¹ and 0.633 mg L⁻¹, respectively.

3.7 Interference study

The interference experiment was carried out to evaluate the effect of some ions commonly found in aquaria on the electrochemical determination of nitrites. The selected interfering ions were: Cl⁻, SO₄²⁻, Ca²⁺, K⁺, Na⁺ and HCO₃²⁻. Indeed, three known concentrations of each selected ions (5, 10 and 15 mg L⁻¹) was added successively to a 10 mg L⁻¹ nitrite solution and the obtained solutions were analysed using DPV technique with the unmodified CPE, and results are presented in [Table 2](#).

Table 2: Effect of interfering ions on the response of CPE to 10 mg L⁻¹ NO₂⁻ 0.1 M in PBS pH 7.4.

Interfering ions	[added ions] (mg L ⁻¹)	% Variation in the DPV peak current (with I of NO ₂ ⁻ = 100%)
Cl ⁻	5	-4.05
	10	-6.79
	15	-9.53
SO ₄ ²⁻	5	-1.92
	10	-3.10
	15	-3.08
HCO ₃ ²⁻	5	-4.2
	10	-4.4
	15	-8.8
Ca ²⁺	5	-4.04
	10	-4.91
	15	-9.51
K ⁺	5	+3.56
	10	+7.70
	15	+9.71
Na ⁺	5	-11.49
	10	-11.88
	15	-10.53

From [table 2](#), it can be observed that all the selected ions slightly interfere on the oxidation signal of nitrites 10 mg L⁻¹. Some ions such as Cl⁻, SO₄²⁻, HCO₃²⁻, Ca²⁺ and Na⁺ interfere by reducing the oxidation signal of nitrites, while only K⁺ ion interferes by increasing the oxidation signal of nitrites. The obtained results come to prove that the proposed sensor could be efficiently used for the determination of nitrite ions in any matrix in the presence of the tested ions.

3.8 Repeatability and reproducibility of CPE

The repeatability of the proposed method consisted to perform a series of experiments on the determination of nitrite ions in the same conditions but at different days. Thus, at each day 1, 3 and 5, three different solutions of nitrites were prepared respectively at 1 mg L⁻¹, 5 mg L⁻¹, 10 mg L⁻¹ and analysed by DPV on the unmodified CPE. The obtained peak currents for each solution at different days are summarised in the [table 3](#). Each analysis was recorded in triplicate.

Table 3: Repeatability analysis for nitrites determination

[NO ₂ ⁻] (mg L ⁻¹)	Peak intensities (μA)					
	Day 1	RSD (%)	Day 3	RSD (%)	Day 5	RSD (%)
1	20.7	4.21	20.8	4.80	21.7	3.32
5	36.6	3.53	37.2	2.76	37.0	4.60
10	71.2	3.14	72.5	4.37	71.8	3.90

From [table 3](#), all the relative standard deviations (RSD) obtained for oxidation peak currents of nitrites were below 5%. These results indicate that the obtained data sets were scattered from 2 to 5% around the mean.

The reproducibility was evaluated by recording DPV measurements in 0.1M PBS containing 10 mg L⁻¹ nitrites on five different unmodified CPE (E₁, E₂, E₃, E₄ and E₅), fabricated individually using the same protocol. Three voltammograms were recorded with each electrode and the obtained results are summarized in the [Table 4](#).

Table 4: Reproducibility analysis for nitrites determination

[NO ₂ ⁻] (mg L ⁻¹)	Peak intensities (μA)									
	E ₁	RSD (%)	E ₂	RSD (%)	E ₃	RSD (%)	E ₄	RSD (%)	E ₅	RSD (%)
70.3	70.3	4.01	70.5	5.38	71.8	5.91	69.9	6.03	70	5.26

E= Electrode

Globally, it can be seen in [table 4](#) that the relative standard deviations (RSD) for the five tested electrodes are in the range from 4% to 6%. This indicates that the elaborated unmodified CPE possesses an acceptable range for reproducibility.

3.9 First analytical applications : water aquarium analysis

The elaborated unmodified CPE was successfully applied for monitoring the levels of nitrites in an aquarium. Thus, two waters samples from an aquarium were collected and analysed using a standard addition method on CPE. [Figure 9](#) shows the differential pulse voltammograms recorded in the sample (curve (a)) and at different concentrations of standard nitrites added (curves b–f).

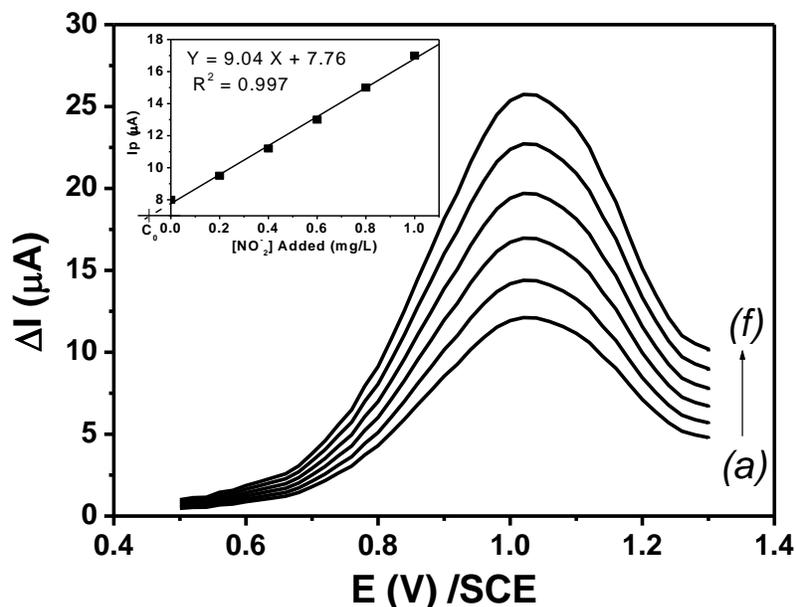


Figure 9: DPV of the CPE recorded for the water sample from an exotic aquarium containing 0.1 M PBS (pH 7.4), upon addition of known amounts of nitrites in the sample. Insert displays the corresponding calibration curve.

The linear relationship was obtained between the peak intensities and the nitrites concentrations (insert of Figure 9), and expressed by the following Equation (3):

$$I_p = 9.04[\text{NO}_2^-] + 7.76 \quad (R^2 = 0.997) \quad (3)$$

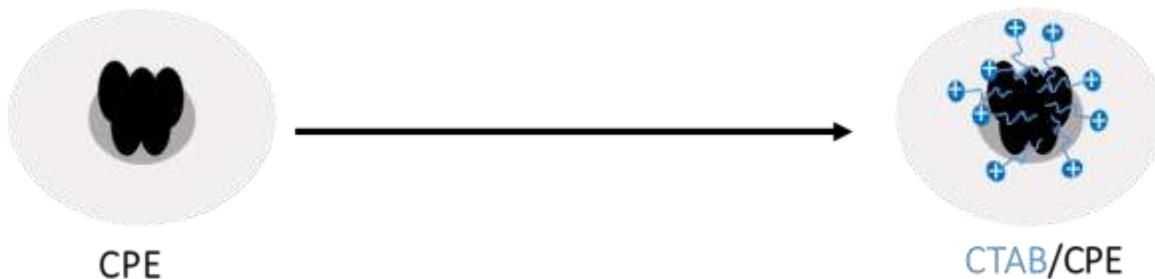
The unknown concentration of nitrites in the solution (C_0) was determined to be $0.88 \pm 0.02 \text{ mg L}^{-1}$ by projection on the abscissa as seen on the calibration curve (insert of Figure 9). The obtained value of 0.88 mg L^{-1} is below the acceptable level of nitrites in aquaria, which is known to be 1 mg L^{-1} . This result come to prove that the proposed method can be efficiently applied for monitoring the level of nitrites in domestic aquaria and we can suggest to test also in aquaculture.

3.10 Improved nitrites sensitivity with modification of the CPE with a surface-active agent dedicated to analysis in rat blood

3.10a- Performances of CTAB/CPE sensor

In this part, the feasibility of improving nitrites sensitivity by creating a better chemical affinity between nitrites and CPE was investigated. To achieve such aim, a monomer of CTAB was

inserted in the carbon paste to create an anionic exchanger with nitrite anions. This idea was inspired from the work reported by Monnappa *et al.* [25].



Scheme 1: Principle of CPE modification with CTAB

A calibration curve was plotted in the concentration range from 0.05 mg L⁻¹ to 0.26 mg L⁻¹ in DPV on the elaborated CTAB/CPE, as shown in Figure 11.

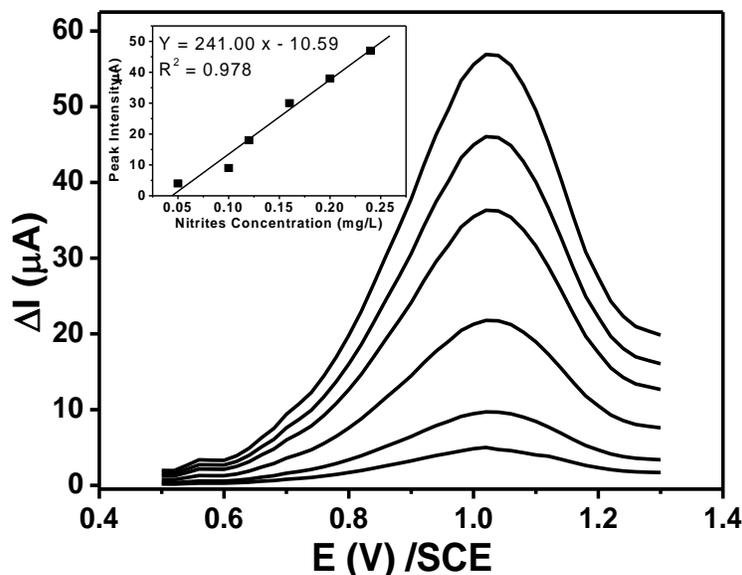


Figure 11: DPV of CTAB/CPE in the presence of (a-f): 0.05, 0.1, 0.12, 0.16, 0.2 and 0.24 mg L⁻¹ nitrites in 0.1 M PBS (pH 7.4). Insert shows peak current versus nitrites concentration

As illustrated in Figure 11, the intensity of the peak current increases linearly ($R^2 = 0.978$) with the concentration of nitrites in the detection medium (inset of the Figure 11) and expressed by the following Equation (4):

$$I_p = 241.00[\text{NO}_2^-] + 10.59 \quad (R^2 = 0.978) \quad (4)$$

A limit of detection (LOD) of 0.034 mg L^{-1} was obtained using the formula $3Sb/m$ where Sb represents the standard deviation and m the slope. The obtained result shows an increasing of slope with a factor 26 when the CPE is modified by CTAB in comparison to the unmodified CPE. This result comes to prove an existing affinity between the surface-active agent CTAB and the nitrite anions due to the anionic exchanger's properties of the modified CTAB/CPE.

3.10b Study of HSA interaction on nitrites analysis with CTAB/CPE

In order to apply the elaborated CTAB/CPE for the determination of nitrite ions in rate blood, we first decided to test the effect of HSA protein on the electrochemical signal of nitrites. Thus, a solution of nitrite 0.2 mg L^{-1} was analysed in the presence and absence of HSA with the modified CTAB/CPE. The obtained results show a passivation of the electrode surface in the presence of HSA, leading to a 30% decrease of the nitrite signal after 3 successive analyses (Figure 12A).

To solve the passivation issue caused by HSA protein adsorption and to recovery the electrochemical signal of the nitrite, the electrode surface was polished after each analysis. Thus, the renewal of the electrode surface led to total recovery of the electrochemical signal of nitrite ions oxidised, as presented in the Figure 12B.

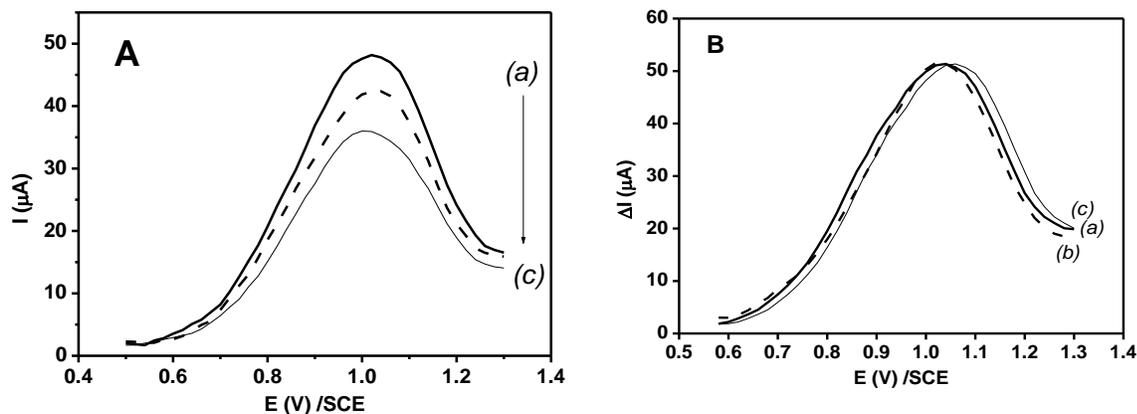


Figure 12: DPV of nitrites at 0.2 mg L^{-1} in presence of 20 g L^{-1} of HAS in 0.1 PBS (pH 7.4) on CTAB/CPE.

The renewal of the CTAB/CPE was therefore optimized and the optimal polishing is to draw 3 lines of 3 cm length after each analysis, as previously optimized and published by Pontie *et al* [27].

3.10 c Blood rat analysis in presence of heparin/lithium

The applicability of the proposed sensor (CTAB/CPE) was its implementation for the quantitative determination of nitrite ions in rat blood. Thus, standard addition method was used under optimum conditions to record differential pulse voltammograms in a solution of rat blood (pH 7.2) as presented in Figure 13.

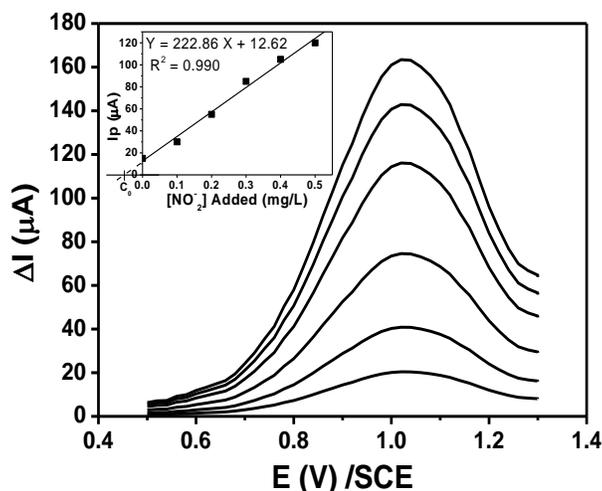


Figure 13: DPV of the CTAB/CPE recorded for the sample of rat blood containing 0.1 M PBS (pH 7.4), upon addition of known amounts of nitrites in the sample. Insert displays the corresponding calibration curve.

From the insert of Figure 13, the relationship between the peak intensities and the nitrite concentrations added is linear ($R^2 = 0.990$) and expressed by the following Equation (5):

$$I_p = 222.86[NO_2^-] + 12.62 \quad (R^2 = 0.990) \quad (5)$$

The unknown concentration of nitrite in the solution (C_0) which represents the exact amount of nitrite in the rat blood was found to be 0.045 ± 0.02 mg L^{-1} (1.02 μM) by projection of linear curve on the abscissa as seen on the insert of Figure 13.

Table 2 compares the obtained analytical performances of the proposed CTAB/CPE sensor for nitrite determination to other sensors reported in recent literature.

Table 5: Comparison of detection limits (LODs) of different modified CPEs reported for nitrites determination

Modified CPE	Limit of detection (μM)	Linear range	pH	References
Ag/HNTs/MoS ₂ -CPE	0.7 μM	2-425 μM	4	[28]
^a [CuII-DHB]-CPE	0.015 μM	2–14 nM	2	[29]
CPE/Ru(III)-BSAP-PLA	1.39 μM	0-0.0138 M	4	[30]
^b Au/PAni/CPE	0.25 μM .	0.038-1.0 M	7	[31]
^c BIBr/CuO/SWCNTs/CPE	0.5 μM	0.004–750 μM	7	[32]
CTAB/CPE	0.64 μM	1-12 μM	7.4	This work

* LOD = 0.0347 mg L⁻¹ (0.64 μM) and LOQ = 0.1159 mg L⁻¹ (2.5 μM)

^aCuII-(N,N'-bis(2,5-dihydroxybenzylidene)-1,2-diaminoethane) (CuII-DHB)

^bgold/polyaniline/carbon paste electrode (Au/PAni/CPE)

^c1-methyl-3-butylimidazolium bromide and CuO decorated single-wall carbon nanotubes (1-M-3-BIBr/CuO/SWCNTs/CPE)

Clearly, the present nitrite sensor denoted CTAB/CPE exhibited an acceptable detection limit and linear range, indicating that this newly developed electrode appears to be a promising result for the direct oxidation of nitrites in human blood and/or plasma in the near future. Further experiments will be engaged for biological interfering studies but presently no ambiguity of nitrites identification and quantification in rat bloods was observed.

Conclusion and perspectives

A hexadecyltrimethylammonium bromide (CTAB) surface active agent was used in this work as an anionic modifier for the elaboration of a CPE dedicated to nitrite ions. The resulting unmodified (CPE) and modified (CTAB/CPE) electrodes were characterized using scanning electron microscopy; optical microscopy and sessile drop techniques that confirmed the presence of the CTAB in the carbon paste. CTAB modified CPE shows a great enhancement with the oxidation peak of nitrites compared to the response obtained using the unmodified CPE. Under optimized

condition using differential pulse voltammetry, a detection limit of 0.034 mg L^{-1} ($0.64 \text{ }\mu\text{M}$) was obtained. The proposed sensor was successfully applied for the determination of nitrites in water aquarium and in rat blood with heparin-lithium. The obtained detection limit shows that the proposed method is a good starting point and should be improved to be suitable for determination of nitrites in the human blood. The intention is to monitor the levels of nitrites in the blood of patients with ischemia diseases. This will be particularly helpful for the early detection of this pathology so that patients should be quickly treated and not be exposed to some chronic lesions.

Data Availability

The original data used in this study can be obtained from the submitting author if requested.

Conflicts of Interest

The authors declare that there are no conflicts of interest.

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