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Full Paper

Determination of Acetaminophen (Paracetamol) by Tranexamic Acid Modified Glassy Carbon Electrode

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Abstract- In this study, tranexamic acid (Txa) (in 0.1 M Sulfuric acid) modified glassy carbon electrode (GC) was tested, for the first time, as a sensor for determining the most consumed pain reliever, paracetamol (ACOP) by differential pulse voltammetry (DPV). The tranexamic acid-modified glassy carbon (rTxa/GC) electrode was characterized by a scanning electron microscope (SEM). Cyclic voltammetry (CV), linear sweep voltammetry (LSV), and electrochemical impedance spectroscopy (EIS) (in aqueous and nonaqueous solution) were used to evaluate the electrochemical performance of electrodes. The modified electrode increased the oxidation peak current of ACOP significantly in this case. The experimental results provide that rTxa/GC electrode displayed excellent electrocatalytic response to the oxidation ACOP. Additionally, the rTxa/GC electrode exhibited excellent electrocatalytic activity for the electrochemical determination of ACOP in Britton-Robinson (BR) buffer solution with pH values ranging from 2 to 12. As a result, the effective electroactive surface area of rTxa/GC electrode increased by using a BR buffer solution with pH 6. The linear range was 25 – 80 μM for ACOP with a limit of detection (LOD) of 4.7 μM and a limit of detection (LOQ) of 14.2 μM.

Keywords- Tranexamic Acid; Acetaminophen; Modified Glassy Carbon Electrode; Differential Pulse Voltammetry; Paracetamol

1. INTRODUCTION

Analgesics and antipyretics, including paracetamol (acetaminophen, ACOP), are widely used throughout the world [1-3]. Among the common uses of ACOP are to relieve joint pain, migraines, and headaches resulting from influenza [4-7]. Through its ability to adjust the body temperature, ACOP controls prostaglandin synthesis and release in the central nervous system [8]. According to research, ACOP administered regularly to humans is usually safe [9]. ACOP has some side effects on the body when taken in excess or continuously [10,11]. A high dosage of ACOP can damage the liver and cause leukemia, as well as malfunctions in the central nervous system.

ACOP is often discharged as industrial waste due to its excessive production [12]. ACOP can pollute the environment when released as industrial effluent [13-15]. As a result, it is important to develop a simple and effective method for determining acetaminophen. The determination of ACOP is widely performed using conventional methods, such as capillary electrophoresis, high-performance liquid chromatography (HPLC), liquid chromatography-mass spectrometry (LC–MS), chemiluminescence, fluorescence spectrum, spectrofluorimetry [4,8-10], and electrochemical methods [16-18].

It is widely used to detect ACOP using conventional methods and techniques; however, they also possess some serious limitations, such as large equipment, expensive determinations, and complex pretreatments [7]. Therefore, conventional techniques cannot detect ACOP in a routine analysis in a rapid or quick manner [18]. Electrochemical sensors have been developing at an incredible rate in recent years due to increasing attention to the electrochemical method-based sensing platform [19-21]. There are many advantages to electrochemical sensing platforms, including fast response, high sensitivity, good selectivity, cheapness, simplicity, and stability [22-28]. The electrochemical oxidation of ACOP allows the detection of ACOP by using electrochemical methods [16,17]. Detection of trace amounts of pharmaceutical compounds is possible by voltammetry, an electrochemical technique that is highly sensitive. Several voltammetry techniques can be used to estimate the redox activity of pharmaceutical compounds, including CV, LSV, and DPV. Pharmaceutical compounds have chemically active functional groups that can undergo redox processes.

Currently, electrochemists developing electrochemical sensors for detecting biomolecules or hazardous compounds are extremely interested in the design and fabrication of an ACOP sensor. ACOP is detected kinetically slowly on GC electrodes such as bare electrodes. By coating the bare GC electrode's active surface with electrochemically active electrode materials, researchers have been able to improve the sensing ability of the bare GC electrode [16,17,29-34].

The importance of Txa is concentrated in that it contains an amine group that can bind to the electrode surface and on the other hand it contains a carboxyl group that can be reduced to

give alcohol. The new modifying surface can be effective in identifying some pharmaceutical compounds.

For accurate detection and quantification of acetaminophen, an ACOP sensor must be designed and manufactured with excellent activity and selectivity.

The purpose of this study was to determine ACOP using an electrochemical method using a GC electrode modified with Txa. This study aims to (i) electrochemically modify Txa onto GC electrodes by CV, (ii) characterize Txa-modified GC electrodes by SEM, CV, and EIS, (iii) to determine if the Txa molecules are bonded to the electrode surface as diffusion-controlled, (iv) to determine pH value of BR buffer solution, (v) to determine whether the newly obtained surface is susceptible to ACOP via DPV.

The importance of using Txa modified GC electrode that detected ACOP and achieved LOD and LOQ at levels of μM. However, based on the results obtained in this work, the DPV method using Txa modified GC electrode may be a useful alternative to the more powerful but more expensive chromatographic-mass spectrometry methods for the determination of ACOP in water samples and drugs.

2. EXPERIMENTAL SECTION

2.1. Chemicals and solutions

The used chemicals were obtained from Fluka (Bucharest, Romania), Merck (Darmstadt, Germany), Sigma-Aldrich (St. Louis, Missouri, USA) and Riedelde Haen (Seelze-Hannover, Germany). Ultra-pure quality water with a resistance of 18.2 MΩ cm was used throughout the experiment. 1.0 mM Txa solution was prepared in H_2SO_4. BR buffer solutions were prepared as in the literature [35,36] CH_3COOH, H_3PO_4 and H_3BO_3 were mixed and dissolved using ultrapure water. By using a digital pH meter, pH adjustments were performed by dropwise addition of 0.1 M/1 M NaOH. ACOP solutions were obtained in BR buffer solutions with different pH.

2.2. Electrodes and apparatus

Our experiments were performed using a GAMRY Reference 600+potentiostat/ galvanostat/ZRA with EIS 300 software from GAMRY Instruments (PA, USA) electrochemical analyzer with BAS (Bioanalytical Systems, West Lafayette, IN, USA) Model MF-2012, with a traditional three-electrode cell, BAS model C3. The reference electrodes were Ag/AgCl/3 M KCl (BAS Model MF-2063) for an aqueous medium and Ag/Ag^+ (10 mM $AgNO_3$) (BAS Model MF-2042) for a non-aqueous medium. The counter electrode was Pt wire. VWR pH 1100L pH meter (PA, USA) was used for pH measurements.

2.3. Electrodes and apparatus

The GC electrode was cleaned and polished according to the literature [22-24]. SEM was applied for the characterization of rTxa/GC layers deposited on the GC electrode surface in Figure 1.

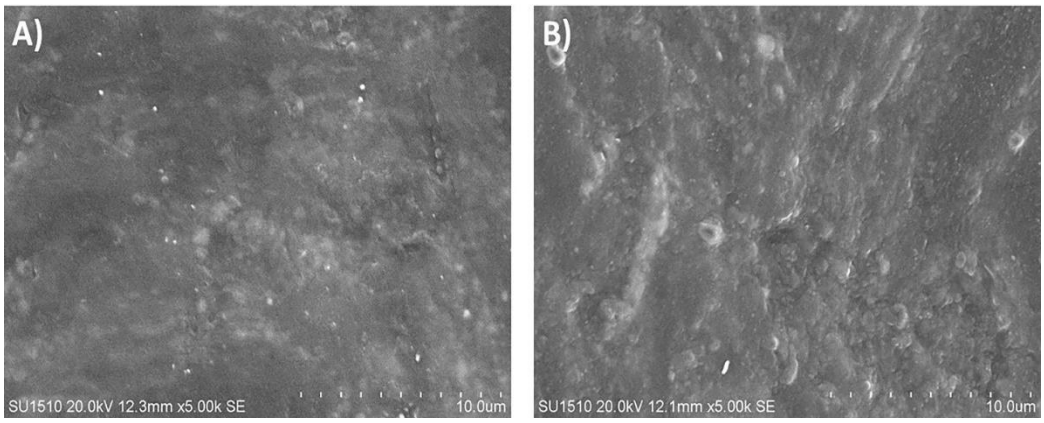


Figure 1. SEM images of **A)** Bare GC and **B)** rTXa/GC electrode

3. RESULTS AND DISCUSSION

3.1. Preparation of modified GC electrode

The GC-modified electrode Txa/GC electrode was prepared by taking 1.0 mM of Txa in 0.1 M H_2SO_4 as supporting electrolyte in aqueous media between +0.5 and +2.0 V for 10 cycles at scan rate of 0.1 V s^{-1}.

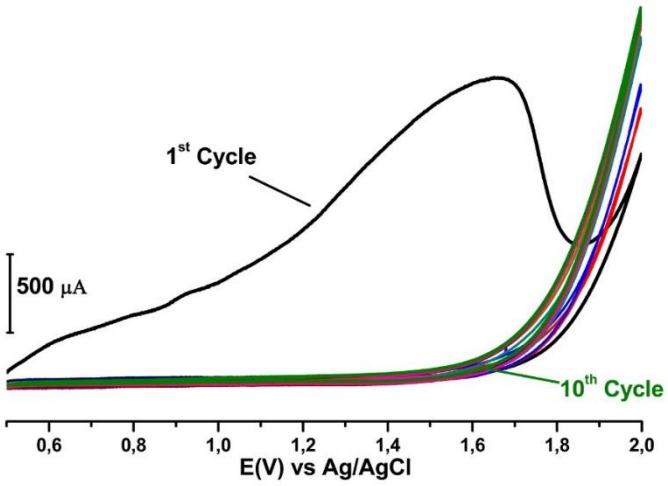


Figure 2. Cyclic voltammograms of preparation of Txa modified GC. 1 mM aqueous solution of Txa (in 0.1 M H_2SO_4) was taken at 10 cycles with the scan rate of 0.1 V s^{-1}

From Figure 2 as can be seen on the cyclic voltammogram, the peak anodic current gradually increases. A peak height becomes more stable once aggregate growth reaches a

saturation level. Therefore, 10 10-cycle modified process was chosen for the fabrication of Txa/GC electrode.

The modified Txa/GC electrode's surface was electro-inactive. In order to activate it, the surface was reduced by CV technique using 0.1 M HCl in the potential range of 0 to -1.2 V with the scan rate of 0.1 V s^{-1} for 5 cycles (Figure 3) (Schem 1). To remove all impurities from the electrode surface, the obtained rTxa/GC electrode was washed, and then it was used for the other experiments in this study.

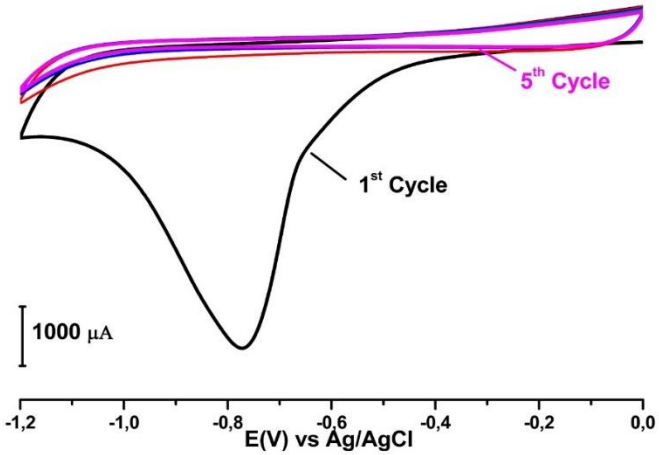
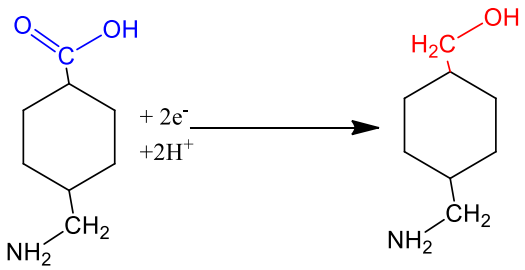


Figure 3. The voltammogram of reduced Txa modified GC electrode was obtained at 5 cycles by using 0.1 M HCl, in the scan rate of 0.1 V s^{-1}



Schem 1. Reduction of Txa

3.2. Characterization of rTxa/GC Electrode

CV and EIS were used to evaluate the electrochemical performance of electrodes. In this study, the modified electrode was studied in a ferrocene redox probe in a non-aqueous solution and $K_3Fe(CN)_6$ in an aqueous solution by using CV. While the modified electrode was only studied in $[Fe(CN)_6]^{3-/4-}$ in aqueous solution by using EIS.

As can be seen in Figure 4A, ferrocene redox probe solution in CH_3CN + 0.1 M NBu_4BF_4 at bare GC electrodes, Txa/GC electrodes, and rTxa/GC electrodes compared to Ag/Ag^+ (10 mM).

In Figure 4B, bare GC, Txa/GC, and rTxa/GC electrodes were used to measure the electrochemical response of 1 mM $K_3Fe(CN)_6$ redox probe solution (in 0.1 M H_2SO_4) *vs.* Ag/AgCl/KCl(sat) reference electrode.

The redox peak pattern of rTxa/GC electrode is well defined, and the peak current of the oxidation and reduction is bigger than that of bare GC and Txa/GC electrodes, indicating a high electrocatalytic activity.

Figure 4C represents typical Nyquist plots for electrochemical impedance spectra in 0.1 M KCl containing 1 mM equivalent molar ratio of $Fe(CN)_6^{3-}$/ $Fe(CN)_6^{4-}$ at 10 mV wave amplitude.

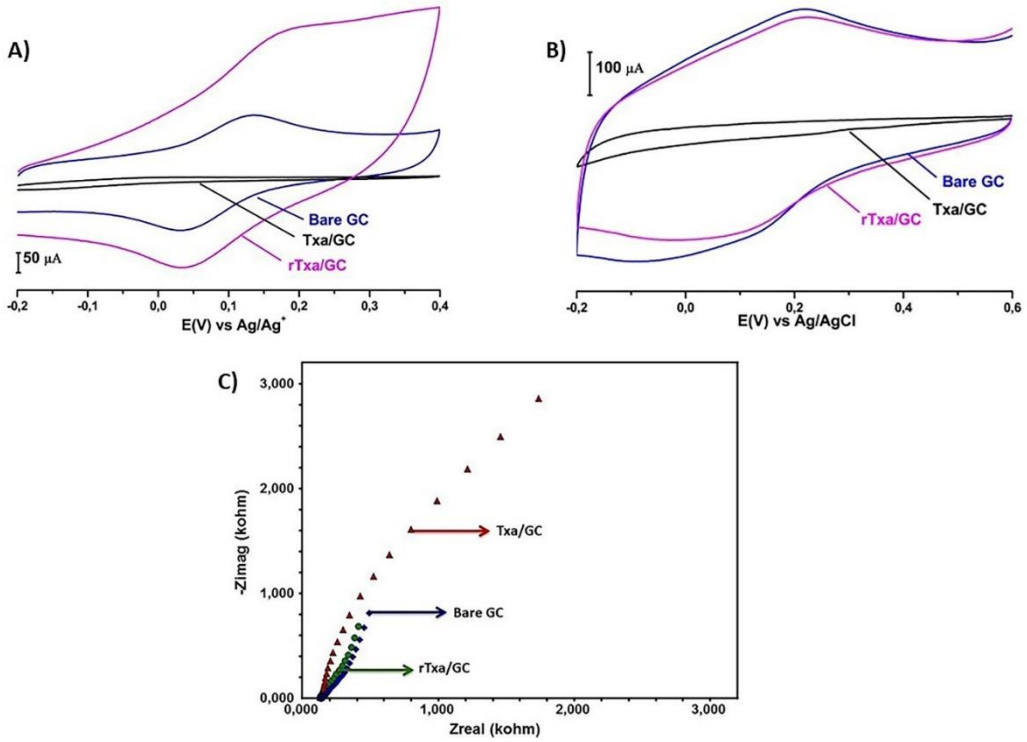


Figure 4. Voltammograms of bare GC, Txa/GC and rTxa/GC electrodes at A) 1 mM ferrocene redox probe solution (in CH_3CN + 0.1 M NBu_4BF_4) vs. Ag/Ag^+ (10 mM) at the scan rate of 0.1 V s^{-1}; B) 1 mM $K_3Fe(CN)_6$ redox probe solution (in 0.1 M H_2SO_4) vs. Ag/ AgCl/KCl(sat) reference electrode at the scan rate of 0.1 V s^{-1}; C) Nyquist plots for electrochemical impedance spectra in 0.1 M KCl containing 1 mM equivalent molar ratio of $Fe(CN)_6^{3-}$/ $Fe(CN)_6^{4-}$ at 10 mV wave amplitude for bare GC, Txa/GC and rTxa/GC electrodes

3.3. Effecting of Scan Rate of Txa at GC Electrode by Using LSV

Scan rate effect on peak currents of Txa (in 0.1 M H_2SO_4) at the GC electrode was studied at 25 to 150 mV s^{-1}. Figure 5 displays the cyclic voltammograms of Txa in 0.1 M H_2SO_4 at the GC electrode at different scan rates from 25 to 150 mV s^{-1}.

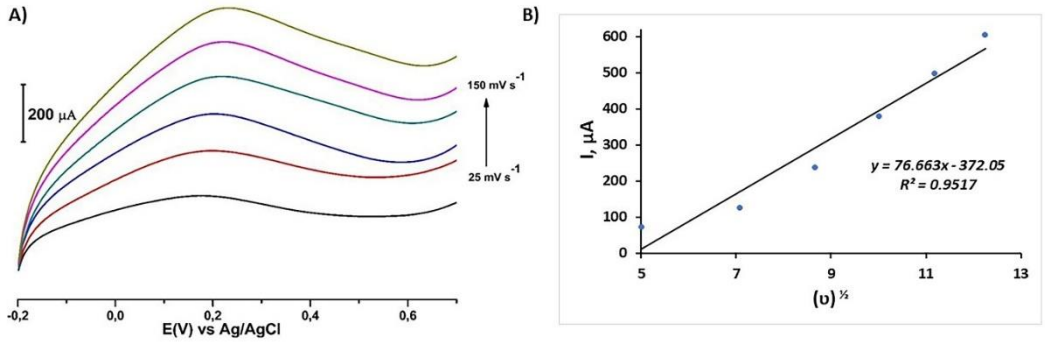


Figure 5. A) Overlaying LSV voltammograms for 1.0 mM Txa (in 0.1 M H_2SO_4) at GC electrode with various scan rates (25–150 mV s^{-1}); B) The linear correlation between the Txa anodic peak currents (µA) and the square root of scan rate (25–150 mV $s^{-1})^{1/2}$

As shown in Figure 5A, the anodic peak shifts as the scan rate increases in Txa oxidation on a GC electrode. This indicates that oxidation is irreversible. As can be seen from Figure 5B, the oxidation peak current of Txa (I_p) scales linearly with the square root of the scan rate ($\upsilon^{1/2}$) in the range from 25 to 150 mV s^{-1}, indicating a diffusion-controlled process.
The graph of I_{pa} vs. the square root of the scan rate is indicated in Figure 5B and the correlation coefficient was 0.9517. The linear regression equation is as follows.

$$I_{pa}(\mu A) = -372.05 + 76.663\ \upsilon^{\frac{1}{2}}(mV\ s^{-1})^{\frac{1}{2}}\ ;\ R^2 = 0.9517$$

3.4. The pH Effect on The Detection of ACOP

In order to detect ACOP, it is important to know the pH of the buffer solution because protons play a key role in electrode reactions.

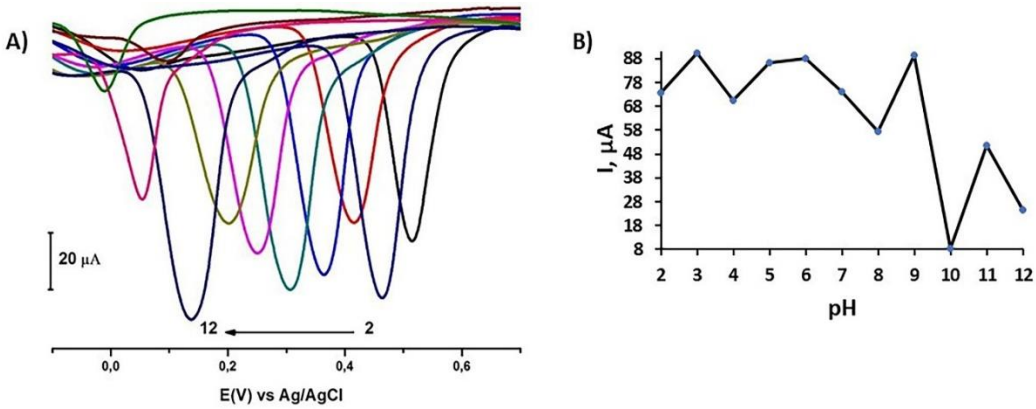


Figure 6. A) Effect of pH buffer solution on the determining of ACOP at the rTxa/GC electrode ~~by~~ using DPV in the potential range ~~of~~ from -0.1 V to +0.7 V with the scan rate of 0.1 V s^{-1}; B) Relationship between peak currents for 1 mM ACOP at the rTxa/GC electrode and pH buffer solution

Figure 6A presents the DPV of rTxa/GC electrode for 1 mM ACOP in BR (0.1 M) at various pH values (2–12) with a scan rate of 0.1 V s^{-1}. The ACOP oxidation peaks are generally more positive when the pH decreases from 12 to 2 (Figure 6A).

The pH against oxidation peak current is shown in Figure 7. At pH 3, 5, 6 and 9 maximum peaks current were observed. The series of concentrations was studied at pH 3,5,6 and 9 to select the best one.

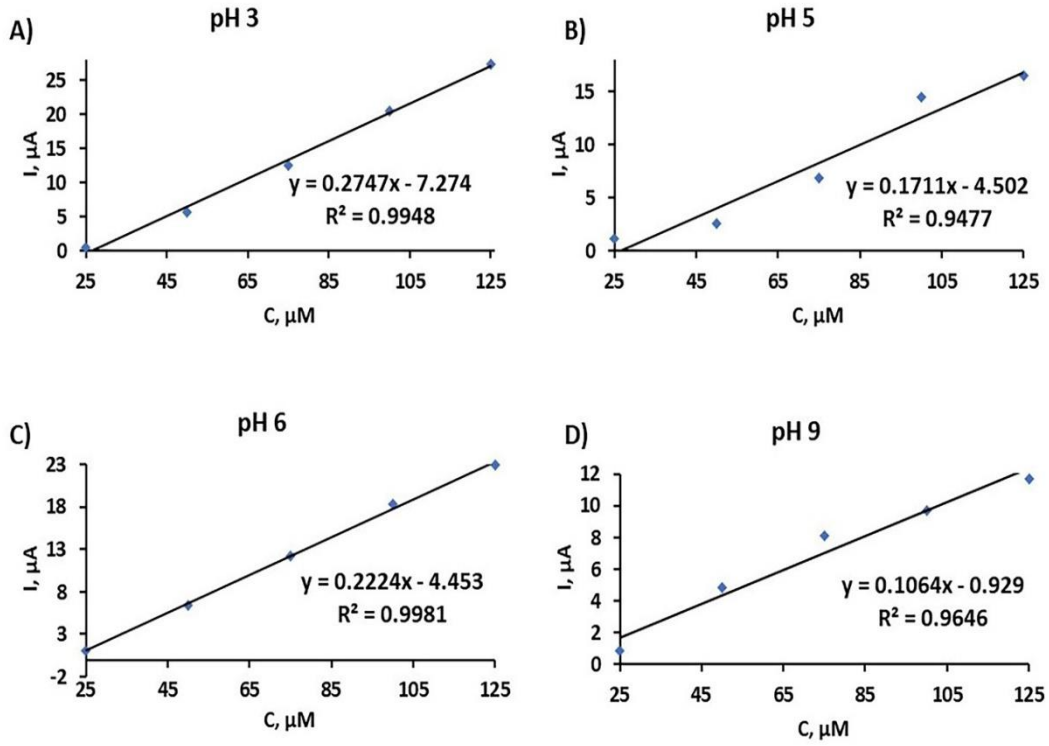


Figure 7. The plot of oxidation peak current versus the concentration of ACOP in different pH values for BR buffer solution with (pH 3, 5, 6 and 9) at the rTxa/GC electrode

From Figure 7, the linear regression equations are as follows:

pH 3 $I_{pa}(\mu A) = -7.274 + 0.2747\ [ACOP]\ (\mu M)\ ;\ R^2 = 0.9948$

pH 5 $I_{pa}(\mu A) = -4.502 + 0.1711\ [ACOP]\ (\mu M)\ ;\ R^2 = 0.9477$

pH 6 $I_{pa}(\mu A) = -4.453 + 0.2224\ [ACOP]\ (\mu M)\ ;\ R^2 = 0.9981$

pH 9 $I_{pa}(\mu A) = -0.929 + 0.1064\ [ACOP]\ (\mu M)\ ;\ R^2 = 0.9646$

$R^2 = 0.9981$ for pH 6 is the best. Therefore, pH 6 was chosen as an ideal pH.

3.5. Electrochemical Investigation of ACOP on rTxa/GC Electrode by DPV

Antipyretic drugs, such as ACOP, are considered to be the most common, important, and commonly used. In order to prevent ACOP poisoning, ACOP doses in serum should be less than 30 mg L^{-1} (200 mol L^{-1}) [37]. Therefore, the calibration curve for ACOP detection was constructed based on a concentration range that corresponds to that of the actual concentration

range of ACOP. In accordance with this, the DPV method was used to investigate the effect of changing ACOP concentrations (Figure 8A). The oxidation DPV peak of ACOP was observed at about +0.44 V on the rTxa/GC electrode. The calibration curve of the ACOP concentrations at rTxa/GC electrode surface was performed for the concentration range of (25×10^{-6} up to 80 $\times 10^{-6}$ M) in 0.1 M BR buffer solution (pH 6) with the scan rate of 0.1 V s^{-1}.

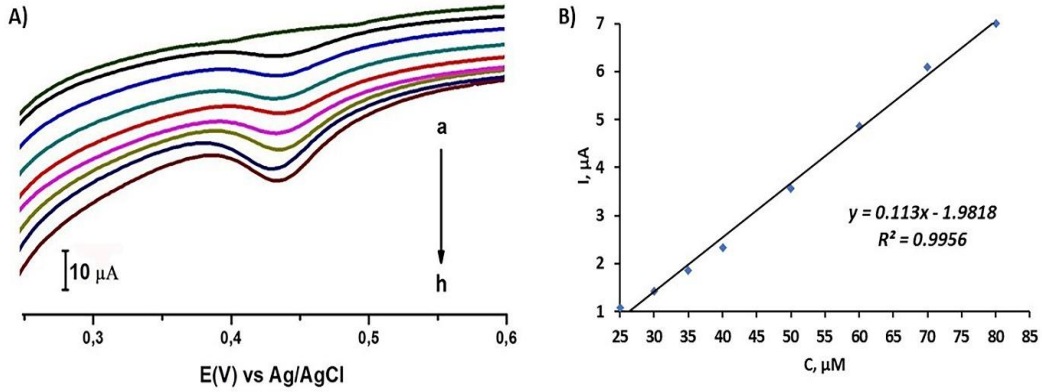


Figure 8. A) DPV curves of ACOP with different concentrations (a) 25 µM, (b) 30 µM, (c) 35 µM, (d) 40 µM, (e) 50 µM, (f) 60 µM, (g) 70 µM and (h) 80 µM in BR buffer solution pH 6.0, B) The plot oxidation peak current versus the concentration of ACOP

According to Figure 8B, the sensor has a linear response as shown below:

$$I_{pa}(\mu A) = -1.9818 + 0.113\,[ACOP](\mu M)\ ;\ R^2 = 0.9956$$

By using the slope (m=0.113) of the calibration curve, we estimated the limit of detection and the limit of quantification. In analytical procedures, the limit of detection determines the minimum amount of analyte in a sample that can be detected [38]. Furthermore, the limit of quantification describes the smallest concentration of drug that can be quantitatively detected using a specified accuracy and precision.

Table 1. Comparison with different modified electrodes for the determination of ACOP in the literature

Electrode	**Technique**	**LOD (µM)**	**Reference**
TiO_2 nanoparticle MCP electrode	CV	5.2	[39]
Bi_2O_3/GC electrode	CV	5.05	[40]
C60-modified glassy carbon electrode	DPV	50.33	[41]
SWCNT/MCP electrode	CV	5.0	[42]
rTxa/GC electrode	DPV	4.7	This work

The limits of detection (LOD) and limits of quantification (LOQ) were calculated for ACOP according to the following equations:

$$\mathrm{LOD} = 3.3\frac{\mathrm{s}}{\mathrm{m}} \qquad \mathrm{LOQ} = 10\frac{\mathrm{s}}{\mathrm{m}}$$

where s is the standard deviation and m is the slope of the calibration curves. For rTxa/GC modified electrode, LOD and LOQ were found to be 4.7 µM and 14.2 µM, respectively.

The comparisons between the rTxa/GC electrode and some reported electrodes for ACOP determination are summarized in Table 1.

4. CONCLUSION

In this work, the analytical performance of rTxa/GC electrode was studied for the determination of ACOP by DPV. Firstly, the potential range and condition media were optimized, achieving the best DPV response in 0.1 M BR buffer solution (pH 6) and scanning the potential from +0.25 to +0.6 V at 0.1 V s^{-1} scan rate. According to the abovementioned conditions, rTxa/GC electrode achieved LOD and LOQ for ACOP at levels of µM. LOD and LOQ for ACOP were 4.7 and 14.2 µM, respectively. Since rTxa/GC electrode has the highest sensitivity and is able to detect very low µM levels of ACOP at low concentrations, it was studied carefully for measuring ACOP in BR pH 6 at low concentrations. Based on the results obtained, the DPV method using rTxa/GC electrode may be a useful alternative to the more powerful but more expensive chromatographic-mass spectrometry methods for the determination of ACOP in water samples and drugs.

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Declarations of interest

The authors declare no conflict of interest in this reported work.

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