

BJP

Bangladesh Journal of Pharmacology

Research Article

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Use of guanine-modified glassy carbon electrode as an electrochemical sensor for the determination of paracetamol

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Article Info

Received: 25 May 2023
Accepted: 19 August 2023
Available Online: 21 August 2023
DOI: 10.3329/bjp.v18i3.66459
Cite this article:
Islamoglu N, Demir Mulazımoglu A. Use of guanine-modified glassy carbon electrode as an electrochemical sensor for the determination of paracetamol. Bangladesh J Pharmacol. 2023; 18: 97-104.

Abstract

In this study, guanine-modified glassy carbon electrode sensor was developed for the determination of paracetamol. The use of overdose continuously for a long time, its metabolites accumulate and affect the kidneys and liver. Therefore, the detection of paracetamol is of great significance. Differential pulse voltammetry and cyclic voltammetry techniques were employed to analyze the behavior of paracetamol in 0.1 M Britton-Robinson buffer solution of pH 5.0 on guanine-modified electrode. Guanine-modified glassy carbon electrode showed a good linear response in the concentration range from 0.5 mM to 10 μ M for the quantitative determination of paracetamol with the lower limit of detection of 0.9 μ M.

Introduction

One of the most commonly used drugs is paracetamol. Overdose of it can lead to the accumulation of toxic metabolites, which may lead to severe and even fatal hepatotoxicity and nephrotoxicity (Bui et al., 2012). Therefore, it is important to develop a method to determine paracetamol.

There are many reports in this area, including titration (Kumar et al., 1997), spectrophotometry (Filik et al., 2009), capillary electrophoresis (Pérez-Ruiz et al., 2005), fluorometry (Al-Ghannam et al., 2002), FIA (Wangfuegkanagul et al., 2002) and HPLC (Nebot et al., 2007). However, these methods also have some disadvantages, including the need for sample pretreatment, high cost, and long analysis time. Spectrophotometry is a simple, rapid, and inexpensive method for the determination of paracetamol, but it has some limitations (Montaseri et al., 2018). The existence of spectral interference caused by other components of the drug, the maximum wavelength of paracetamol is 244 nm, which can be interfered with propyphenazone (266 nm) and

caffeine (273 nm) (Delvadiya et al., 2013). Therefore, methods for the determination of paracetamol in pharmaceuticals need more selective and sensitive methods.

For this purpose, a chemical-modified electrode can be modified with guanine. The chemical-modified electrode is coupled to an adapter that converts its interaction with the analyte into a measurable electrochemical signal (Boumya et al., 2021).

Materials and Methods

Chemicals and Reagents

All reagents used for the development of this work were of analytical grade, obtained from Merck, Fluka, Riedel, and Sigma-Aldrich, including the pharmaceuticals paracetamol and guanine. All the chemicals were used without any purification. All solutions of used chemicals in characterization were prepared as 1 mM in non-aqueous medium; 100 mM tetrabutylammonium tetrafluoroborate (NBu₄BF₄) in acetonitrile (CH₃CN).



The employed BR buffer solutions were prepared by mixing H_3PO_4 , H_3BO_3 , and CH_3COOH according to preparation conditions in the literature, completed to the level by using ultrapure water. The pH adjustments were carried out by dropwise addition of 0.1 M/1 M NaOH by a digital pH meter. All guanine solutions used in modification were 1 mM concentration in 0.1 M H_2SO_4 solution. The modification was carried out only in aqueous media, while the electrochemical characterization was done in both non-aqueous and aqueous media.

Instruments

Ultrapure water with a resistance of 18.2 M Ω cm (MP minipure purification system, USA) was used to preparation all aqueous solutions, measurements, cleaning of the glassware, and polish the electrodes.

All electrochemical experiments were completed at room temperature ($25 \pm 1^\circ\text{C}$) under a traditional three-electrode cell system for all electrochemical experiments.

pH meter with a combined glass pH electrode (VWR pHenomenal) was used to measure the pH value in the aqueous solutions.

CV and electrochemical impedance spectroscopic (EIS) techniques were performed using a GAMRY reference PCI4/Series 750 and Reference 600+ Potentiostat/Galvanostat/ZRA from GAMRY Instruments (USA) equipped with a BAS (Bioanalytical Systems, USA) model C3 cell stand.

Ag/AgCl/KCl (sat) (BAS Model MF-2063) reference electrode was used in aqueous solutions. Ag/Ag⁺ (10 mM AgNO₃) (BAS Model MF 2042) in 0.1 M NBu₄BF₄ in CH₃CN reference electrode was used in non-aqueous solutions. A platinum wire (BAS Model MW-1033) was used as a counter electrode. Bare GC electrode with a geometric area of 0.071 cm² or guanine-modified GC electrode BAS (Bioanalytical Systems, USA) model MF-2012 and the Tokai GC-20 (Japan) GC electrode were used as a working electrode in all electrochemical experiments such as CV, EIS and DPV. The GC electrode was cleaned and polished with 100 and 50 nm Al₂O₃ suspension (Baikowski Int. Corp., USA) on polishing pad (Buehler, USA) for about 5 min and then washed with ultrapure water (UPW). Polished GC electrodes were sonicated (Bandelin electronic, RK100H, Germany) in UPW for about 5 min in the following sonication GC electrodes were dipped in CH₃CN media. All working electrodes were kept in CH₃CN when they were not in use in all experiments.

Electrode preparation and modification

The GC electrodes were prepared for the experiments by polishing to gain a mirror-like appearance, first with fine wet emery papers (grain size 4000) and then with 1.0 μm and 0.3 μm alumina slurry on micro-cloth pads

(Buehler, USA). After the initial polishing, the GC electrodes were resurfaced with 0.05 μm alumina slurry. First, in the following order, the GC electrodes were sonicated both in water and in CH₃CN for 10 min, (Demir Mulazimoglu and Mulazimoglu, 2013). Electrochemical surface modification experiments were performed in the +0.5 and +1.7 V potential range at a 0.1 V s⁻¹ scan rate with 20 cycles. Then reduction experiments were achieved by using 0.1 M HCl solution in the 0 and -1.2 V potential range at a 0.1 V s⁻¹ scan rate with 5 cycles. After the modification of the GC electrode, the surface of the obtained rGu/GC electrode was washed to remove all impurities from the electrode surface, and it was then used for other investigations described in this study.

Results

Modification of GC electrodes

Electrode modification plays an important role in electrochemical research and has been widely used in the past decade. These modified electrodes have achieved remarkable results in the determination of organic and inorganic substances. Since the electrode surface can be prepared by electrochemical oxidation or reduction in a suitable medium and under optimal preparation conditions, modified electrodes can be physically prepared to get a sensor. Although electrode modification is widely performed in aqueous media, recent studies can also be applied to non-aqueous media. Modification methods in aqueous media are preferred since assays in research are often performed in aqueous media, which are considered to be more stable.

The electrochemical modification on the surface of the GC electrodes was achieved with 1 mM guanine in 0.1M H_2SO_4 as supporting electrolyte in aqueous media from +0.5 V to +1.7 V potential ranges using 0.1 V s⁻¹ sweep rate with 20 cycles (Figure 1A).

The modified surface was electro-inactive. So, the surface was activated by reducing it in the 0 and -1.2 V potential range using 0.1 V s⁻¹ scanning rate with 5 cycles (Figure 1B).

After that, the surface of obtained rGu/GC electrode was washed to remove all impurities from the electrode surface and then it was used for other investigations described in this study.

Electrochemical characterization of rGu/GC electrode

As the surface characterization processes after modifications and reduction were performed electrochemically by using ferrocene redox probe in non-aqueous medium and Fe(CN)₆³⁻ in aqueous medium with CV. For the characterization processes, it was made with ferrocene prepared by dissolving in 0.1 M NBu₄BF₄. A voltammogram with a potential range from +0.2 V to

+0.6 V and a sweeping rate of 0.1 V s⁻¹ was achieved (Figure 2A). In addition to that, characterization also has been done with Fe(CN)₆³⁻ that was prepared by

letting it dissolve in BR buffer solution, pH 2.0. A voltammogram with the potential range from +0.4 V to 0 V at a 0.1 V s⁻¹ sweep rate was carried out (Figure 2B).

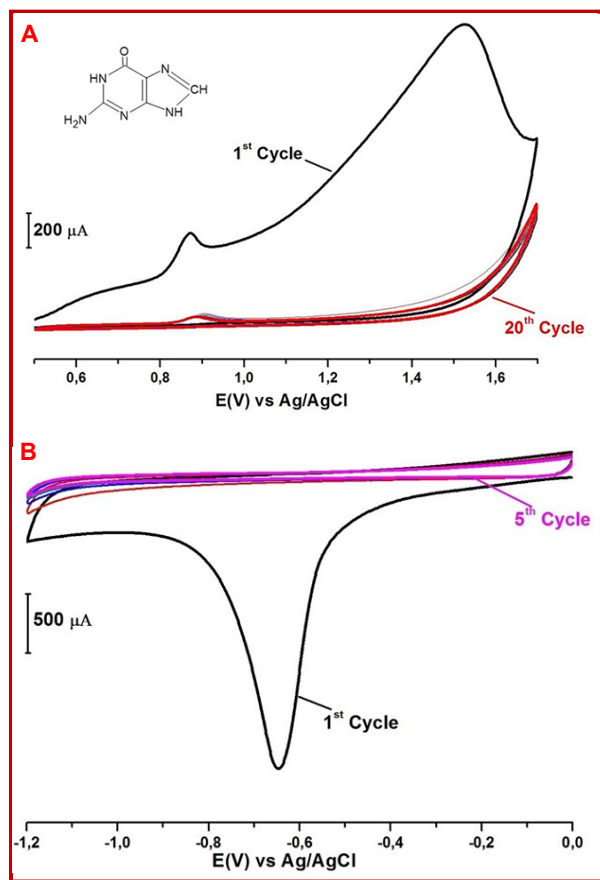


Figure 1: Cyclic voltammograms of preparation of guanine modified GC electrode, 1 mM aqueous solution of guanine was taken in 0.1 M H₂SO₄ at 20 cycles with scan rate 0.1 V s⁻¹ (A). Cyclic voltammograms of reduction of guanine modified GC electrode, 0.1 M HCl was taken at 5 cycles with scan rate 0.1 V s⁻¹ (B)

Effect of buffer solution pH on the sensor response

The pH value of the electrolyte can affect the peak shape, peak potential, and peak current of the modified electrode. It is also more conducive to estimating the ratio of protons and electrons participating in the electrode reaction. As shown in Figures 3A, 3B, it was investigated the effect of pH values using BR buffer solutions from 2.0 to 12.0 at a scan rate of 0.1 V s⁻¹.

The observed peak potential shifted in the negative direction with increasing pH, confirming the direct involvement of protons in the rate-limiting step. Comparing the peak currents of paracetamol, the maximum response was observed at pH 5.0. Therefore, the same pH was chosen for further determination.

Effect of scan rate on CVs of paracetamol at rGu/GC electrode

The effect of scan rate on the electrochemical behavior of paracetamol was investigated by CV in BR of pH 5.0. Figure 4A shows the CVs of 1.0 mM paracetamol for BR pH 5.0 at different scan rates from 25 to 500 mV s⁻¹.

For oxidation and reduction processes, the peak potentials shift towards more positive and negative values, respectively, indicating that both peak potentials (E_{pa} and E_{pc}) are a function of the scan rate. The oxidation and reduction peak currents (I_{pa} , I_{pc}) increase linearly with the square root of the scan rate ($v^{1/2}$) over the examined range.

$$I_{pa}(\mu A) = -81.094 + 27.507 v^{1/2}(\text{mV s}^{-1})^{1/2}$$

$$R^2 = 0.9937$$

$$I_{pc}(\mu A) = 69.65 - 19.061 v^{1/2}(\text{mV s}^{-1})^{1/2}$$

$$R^2 = 0.9935$$

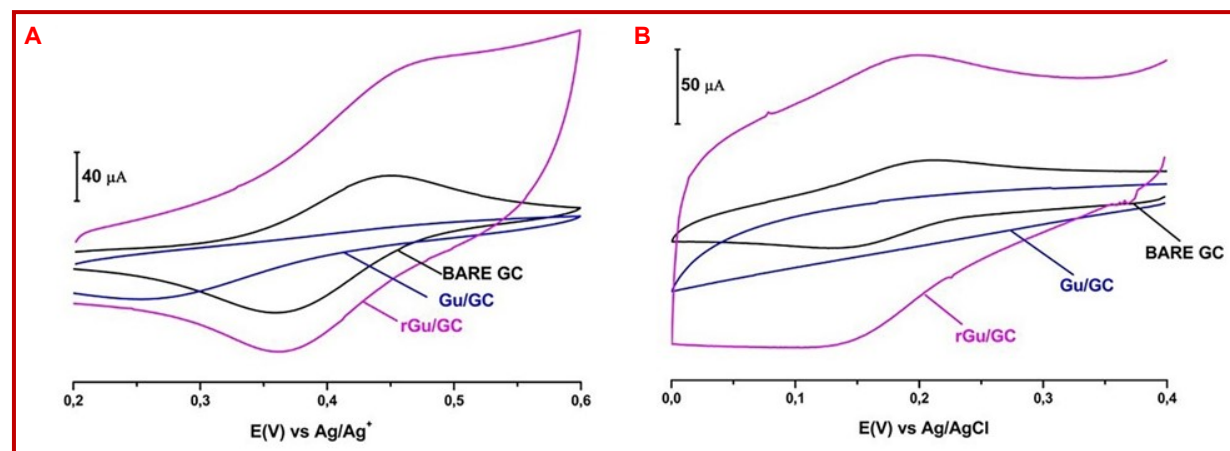


Figure 2: Overlaying the bare GC and rGu/GC electrode voltammograms at ferrocene and K₃Fe(CN)₆. A) 1 mM ferrocene redox probe solution vs. Ag⁺/Ag⁺ (10 mM) in CH₃CN+ 0.1 M NBu₄BF₄ using 0.1 V s⁻¹ scanning rate and B) 1 mM K₃Fe(CN)₆ redox probe solution vs. Ag⁺/AgCl/KCl(sat) reference electrode in BR buffer solution, pH 2.0 using 0.1 V s⁻¹ scanning rate

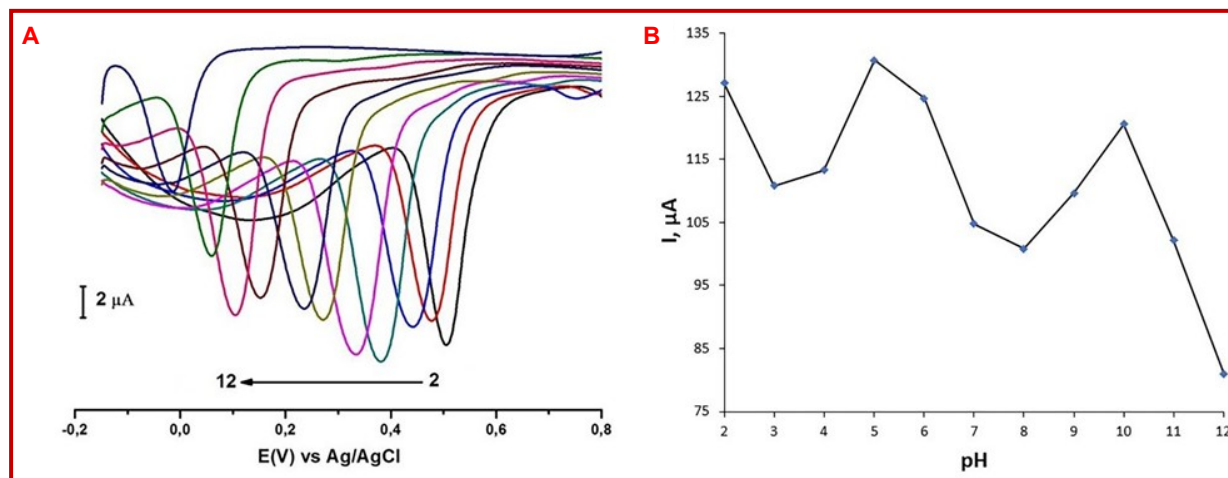


Figure 3: A) Effect of pH values use of BR buffer solutions from 2.0 to 12.0 to determine paracetamol at rGu/GC electrode by DPV from -0.2 to +0.8 V using 0.1 V s⁻¹ scanning rate, and B) Relation between pH and peak currents for 1 mM PAR at rGu/GC electrode

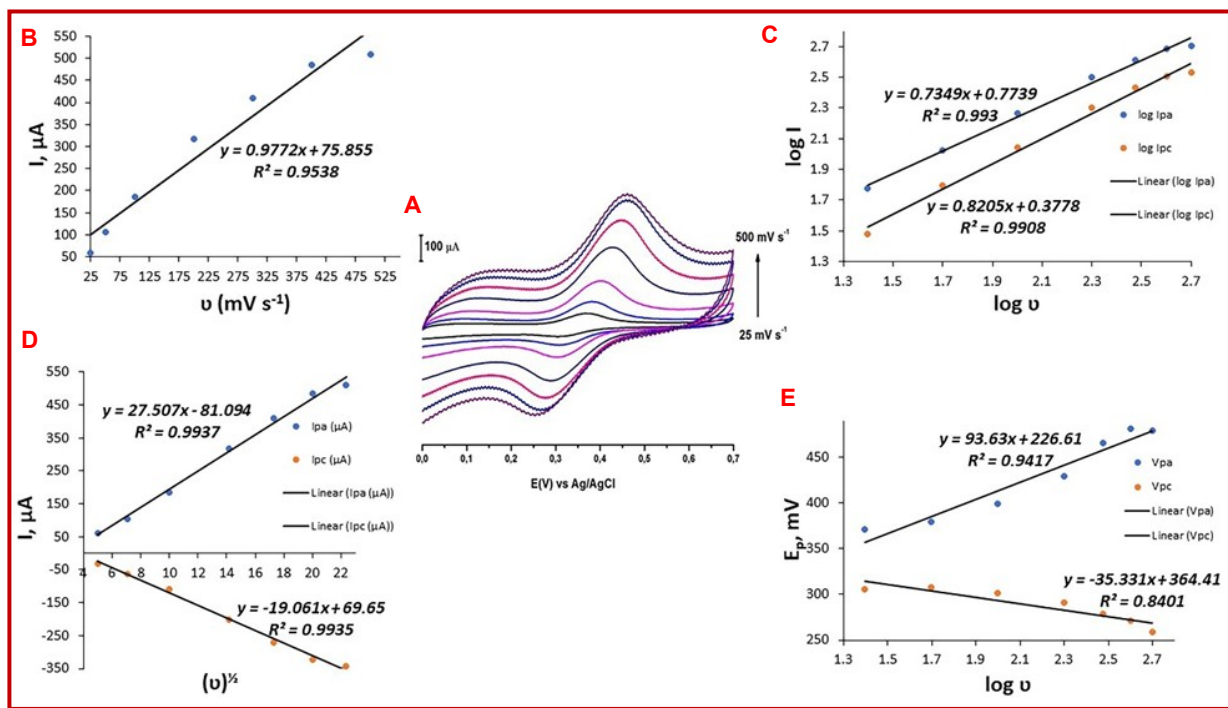


Figure 4: A) CVs obtained at rGu/GC electrode in BR pH 5.0 containing 1.0 mM paracetamol at various scan rates (25-500 mV s⁻¹), B) shows the plot of the anodic peak current versus scan rate. C) Relation between log scan rate and log peak current. D) Calibration plot of the redox peak currents vs. square root of scan rate. E) Plot of variation of E_p, mV versus log ν, mV s⁻¹

These phenomena suggest that the electrochemical reaction is a diffusion-controlled process. On the other hand, a linear regression line plot of the logarithmic peak current and the logarithmic scan rate also provides information about the process controlled by diffusion or adsorption.

Two linear relationships were obtained (Figure 4C) and represented by the following equation:

$$\log I_{pa} = 0.7349 \log \nu + 0.7739; R^2 = 0.993$$

$$\log I_{pc} = 0.8205 \log \nu + 0.3778; R^2 = 0.990$$

The plot of peak potential (E_{pa} and E_{pc}) versus log ν is shown in Figure 4E, and the corresponding regression equation is:

$$E_{pa} = 93.63 \log \nu + 226.6; R^2 = 0.9417$$

$$E_{pc} = -35.331 \log \nu + 364.41; R^2 = 0.8401$$

Effect of scan rate on LSV of paracetamol at rGu/GC electrode

From Figure 5A, for oxidation processes, the peak potentials shift towards more positive values, indica-

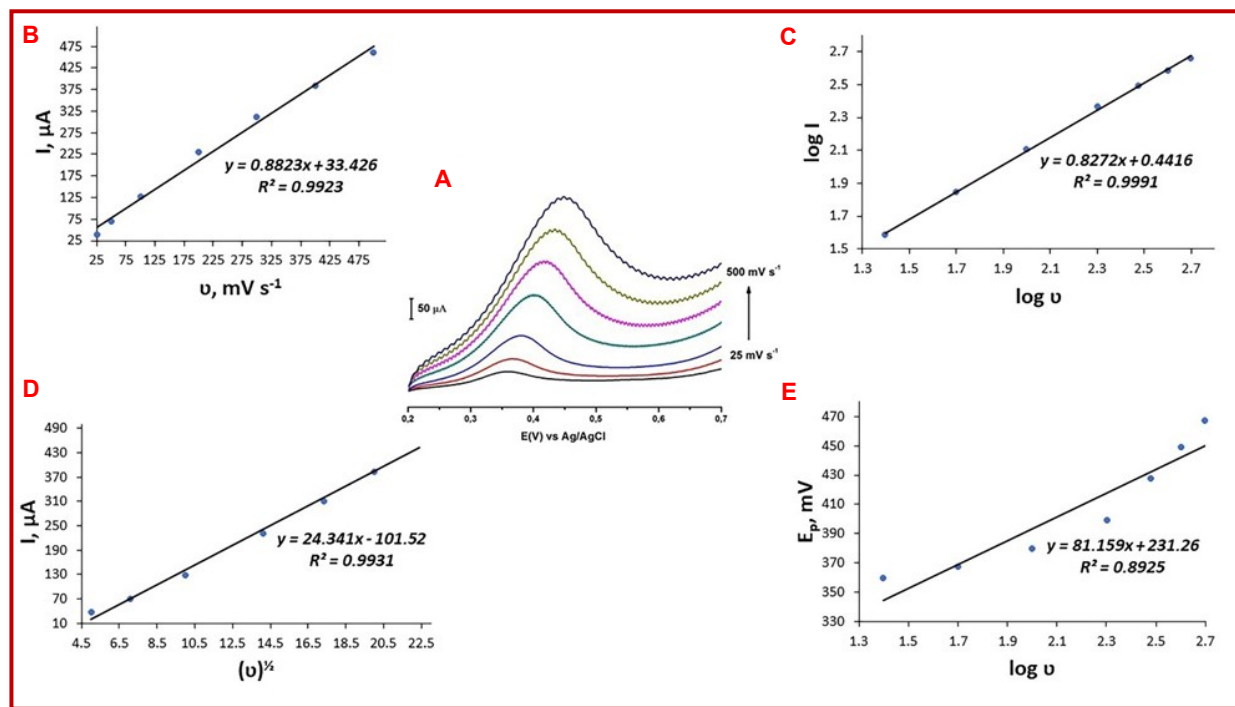


Figure 5: A) Impact of scan rate on 1.0 mM paracetamol in BR pH 5.00 at rGu/GC electrode from 20-500 mV s^{-1} , B) shows the plot of the anodic peak current versus scan rate. C) Relation between \log scan rate and \log peak current. D) Calibration plot of the redox peak currents vs. square root of scan rate. E) Plot of variation of E_p , mV versus $\log \nu$, mV s^{-1}

ting that peak potentials (E_{pa}) are a function of the scan rate. As shown in Figure 5D, the oxidation peak currents (I_{pa}) increase linearly with the square root of the scan rate ($\nu^{1/2}$) over the examined range.

$$R^2 = 0.9931$$

The linear relationship was obtained (Figure 5C) and represented by the following equation:

$$\log I_{pa} = 0.8272 \log \nu + 0.4416; R^2 = 0.9991$$

$$I_{pa}(\mu\text{A}) = -101.52 + 24.341 \nu^{1/2} (\text{mV s}^{-1})^{1/2}$$

The plot of peak potential (E_{pa}) versus $\log \nu$ is shown in Figure 6E, and the corresponding regression equation is:

$$E_{pa} = 81.159 \log \nu + 231.26; R^2 = 0.8925$$

Voltammetric determination of paracetamol

Compared with conventional CVs, DPV has advantages of higher sensitivity and high resolution in quantitative analysis. Therefore, DPV was used to determine paracetamol in 0.1 M BR from 0 to +0.6 V using 0.1 V s^{-1} scanning rate as shown in Figure 6.

The oxidation experiments were performed under optimal conditions. The results of DPV are shown in Figure 6A. The oxidation peak current of paracetamol increases linearly with increasing concentration. Figure 6B shows the linear relationship from 0.5 mM to

10 μM using the following linear equation:

$$I_{pa}(\mu\text{A}) = 0.0931 \mu\text{M} \times [\text{paracetamol}] + 0.3688$$

$$R^2 = 0.9904$$

The limit of detection (LOD) was 0.9 μM , and the limit of quantification (LOQ) was 2.7 μM .

Discussion

Guanine and adenine are important components of DNA (Wang et al., 2002). The electrode surface can be prepared by electrochemical oxidation or reduction in a suitable medium under optimal preparation conditions. Although electrode modification is widely used in aqueous media. Our previous studies (Mulazimoglu and Demir Mulazimoglu, 2012; Demir Mulazimoglu and Mulazimoglu, 2013), demonstrated electrode modification with amine-containing compounds. The main purpose objective of this study was to improve a new chemical sensor electrode for the determination of paracetamol by using electrochemical and spectroelectrochemical techniques. (Demir Mulazimoglu and Mulazimoglu, 2013; Dundar et al., 2011; Celik et al., 2020). The main target of this study was achieved to electrochemically modify guanine onto the GC electrode surface by using cyclic voltammetry (CV) and characterize Gu-modified GC (rGu/GC) electrode by CV. This study also was performed to investigate whether the new sensor obtained is sensitive to the

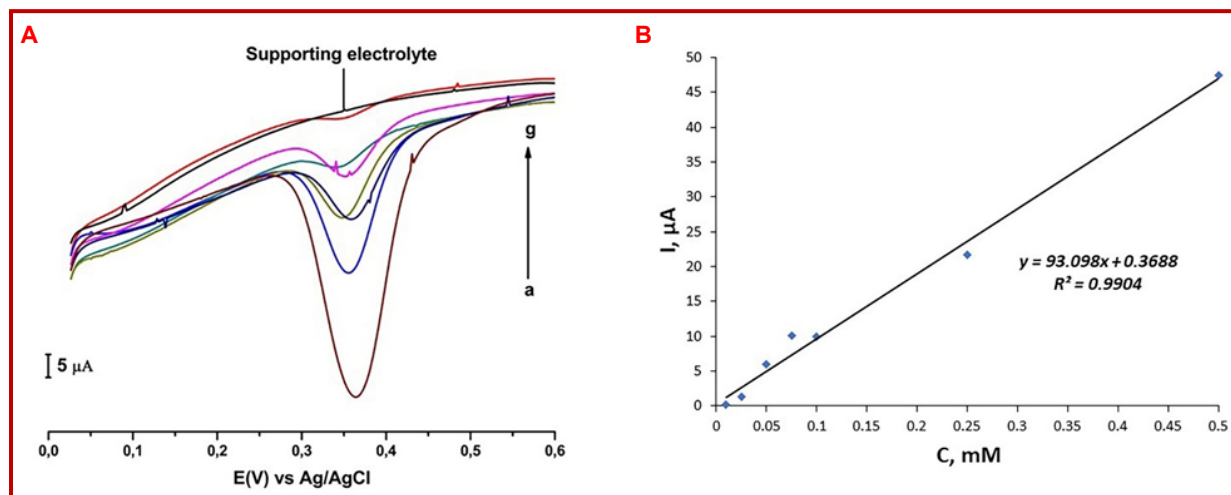


Figure 6: A) DPV curves of paracetamol on its concentration in the range of a) 5.0×10^{-4} M, b) 2.5×10^{-4} M, c) 1.0×10^{-4} M, d) 7.5×10^{-5} M, e) 5.0×10^{-5} M, f) 2.5×10^{-5} M, g) 1.0×10^{-5} M in BR buffer solution pH 5.0, and B) The plot of I_{pa} versus concentration of paracetamol

paracetamol through differential pulse voltammetry (DPV).

Recently, electrochemical methods have attracted much attention due to their simplicity, low cost, high sensitivity, and the possibility of miniaturization (Beitollahi et al., 2013; Esfandiari Baghnamidi et al., 2013; Mohammadzadeh et al., 2018; Mohammadi et al., 2019). Electrochemical techniques are of great significance in environmental monitoring, medicine and biotechnology, and industrial process management (Mohammadi et al., 2021). Paracetamol is an electroactive compound that can be detected using voltammetry. Voltammetry has high sensitivity, high precision, low cost, low detection limit, and wider linear range (Ejaz et al., 2017). The voltametric determination of paracetamol is widely used in blood samples, water, drugs and mixed compounds. Results showed recoveries of 97-102%, with highly reproducible responses (Ozcan et al., 2007; Akbari et al., 2018; Berto, 2018; Tanuja et al., 2018).

Among them, carbon-based electrodes are widely used in voltammetry because of their low cost, wide potential window, low resistivity, and versatility of chemical modification. Chemically modified electrodes are very interesting tools for the analysis of various trace substances using sensitive electroanalytical techniques. In this case, it is very important to choose the most suitable modifier for each analyte because the sensitivity and selectivity of the electroanalytical reaction depend on the nature of the modifier (Barreto et al., 2023; Liu et al., 2023; Siavashi et al., 2023; Smajdor et al., 2023). Modification of carbon electrodes with organic molecules can improve the sensitivity and selectivity of paracetamol determination. The presence of specific modifier molecules on the surface of the electrode allows only certain molecules to diffuse to its surface.

In this work, by using paracetamol as test analytes, the

effectiveness of using modified carbon-based electrodes as an inexpensive and simple in pharmaceutical analysis is evaluated (Akca et al., 2022; Gashu et al., 2022; Kablan et al., 2022; Kassem et al., 2022; Ozkan et al., 2022; Budak et al., 2023; Mohamed et al., 2023).

The test analytes were determined individually by differential pulse voltammetry using guanine-modified glassy carbon electrode. Under optimized experimental conditions (BR buffer solution pH 5.0), the electrode showed a linear response in the range of 0.5 mM to 10.0 μM for quantitative determination of paracetamol with lower limit of detection of 0.9 μM. In this research, advantages were taken of its efficiency, availability, and low cost, avoiding complex, time-consuming. This study provides a reliable and economical basis for the development of low-cost, biodegradable electrode materials for electrochemical sensors. The technology can be used more than once sensor device, similar to a glucose sensor (Sun et al., 2022; Yang et al., 2022; Youcef et al., 2022; Zainul et al., 2022), to measure and quantify paracetamol.

Produced electrodes were tested for acceptable reproducibility. Ultimately, the practical application of the developed sensor can be successfully used for the quantitative measurement of paracetamol in drug and serum samples (Hussain et al., 2023; Nigussie et al., 2023; Qomi et al., 2023; Richard et al., 2023; Uzun et al., 2023). Therefore, this strategy is expected to provide a simple and novel approach for sensor fabrication for the determination of paracetamol.

Conclusion

The rGu/GC electrode presented an acceptable analytical performance for paracetamol determination with a

wide linear range, from 0.5 mM to 10 μ M with the limit of detection of 0.9 μ M.

Financial Support

Self-funded

Conflict of Interest

Authors declare no conflict of interest

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