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RESEARCH ARTICLE

A modified carbon paste based electrochemical sensor for measuring pharmaceutical molecules

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ABSTRACT

Antiviral drugs are critical for treating viral diseases, demanding rapid, accurate, and cost-effective analytical methods for pharmaceutical and biological samples. Conventional techniques like HPLC are accurate but require expensive instrumentation, time-consuming procedures, and high-purity solvents. Electrochemical methods offer simplicity, high sensitivity, rapid analysis, low cost, and portability. In this study, a carbon paste electrode-based electrochemical sensor was developed for favipiravir determination. Parameters including pH, supporting electrolyte, pulse amplitude, duration, and potential step were optimized. Under optimized conditions the reduction process showed maximum response. A strong linear relationship between cathodic peak current and drug concentration was observed from 1 to 600 µM with a correlation coefficient of approximately 0.99. Two linear ranges were noted for oxidation. Detection limits were 0.35 µM (reduction) and 1.55 µM (oxidation), respectively. The sensor exhibited good sensitivity, selectivity, repeatability, and a wide linear range without requiring complex surface modification or expensive nanomaterials. Due to its simplicity, low cost, and satisfactory performance, this method holds strong potential for pharmaceutical quality control, therapeutic drug monitoring, and the development of portable electrochemical sensing systems.

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INTRODUCTION

Drugs are a large and diverse group of organic compounds that exhibit high biological activity at low concentrations and are used for the diagnosis, treatment, or prevention of diseases and other abnormal conditions. Considering the continuously increasing unauthorized and uncontrolled use of pharmaceuticals, the resulting consequences have raised serious public health concerns [1]. The synthesis, formulation, and production of various chemical drugs, along with the widespread availability of many of them without prescription, the high cost of raw materials, and insufficient regulatory control over their distribution, together with the adverse effects of extensive self-medication, collectively highlight the need for rapid and simple methods for the identification of active pharmaceutical ingredients and their residues in biological samples [2]. The increasing regulatory requirements imposed on the pharmaceutical industry have further emphasized the importance of developing advanced drug analytical methods. In

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hospitals, drug analysis is also essential for clinical testing, pharmacokinetic studies, therapeutic drug monitoring, and the assessment of drug abuse in patient samples [3].

In addition, the intentional or unintentional discharge of industrial, hospital, and domestic wastewater has led to the release of pharmaceutical residues into the environment. Furthermore, excessive use of pharmaceuticals in the livestock industry may result in drug residues exceeding permissible limits in animal-derived products [4]. In response to these concerns, the World Health Organization and the Food and Drug Administration have established permissible limits for drug residues in biological fluids (such as blood serum and urine), food products of animal origin, and wastewater. Therefore, there is no doubt that appropriate analytical methods must be employed to determine pharmaceutical substances or active ingredients in various biological matrices [5]. Given these considerations, alongside advances in analytical science and laboratory instrumentation, researchers have long recognized the importance of monitoring drugs in blood and other biological samples for clinical studies and for minimizing adverse effects. Consequently, numerous reports have been published on the determination of pharmaceutical compounds in different matrices [6]. Instrumental methods commonly used for quantitative drug analysis in pharmaceutical laboratories are generally classified into four main categories: chromatographic, spectrophotometric, electrochemical, and radiometric techniques [7]. High-performance liquid chromatography (HPLC) is widely regarded as the standard method for the determination of favipiravir. However, due to the retention time-based separation mechanism in chromatographic columns, the analytical speed is relatively limited, and interfering species with similar retention times may affect the accuracy of measurements in real samples [8]. Moreover, this technique requires high-purity solvents and consumables, which increases operational cost and may introduce additional sources of analytical interference.

Electrochemical sensors offer several advantages, including high sensitivity, linear response, and operational simplicity [9]. Electrochemical methods and biosensors are considered cost-effective, rapid, and reliable alternatives to conventional analytical techniques. However, in HPLC-based methods, the retention time dependency reduces analysis speed, and matrix interferences in real samples can compromise accuracy [10]. Furthermore, the requirement for high-purity reagents and solvents increases cost and may lead to additional analytical complications. In contrast, electrochemical methods are now recognized as fast, simple, low-cost techniques with high selectivity and reproducibility, and they occupy a prominent position among analytical approaches [11]. A wide range of environmentally friendly electrochemical sensors that meet these requirements are commercially available [12]. However, the diversity of analytes and the need for accurate, time- and cost-efficient measurements—particularly in situations where sensor reuse is not feasible and instrument portability and reagent storage are challenging—have driven researchers toward the development of disposable sensors. Accordingly, in situ electrochemical measurements using compact and portable devices have attracted considerable attention [13].

The application of various electrochemical techniques using appropriate electrodes has been proposed as an effective strategy. In recent years, the use of different types of electrodes, including mercury electrodes, solid electrodes, and modified electrodes, has increased for the electroanalysis of electroactive compounds. Carbon has been extensively used as an electrode material due to its desirable properties, such as availability in various structural forms, good electrical conductivity, high mechanical and thermal stability, a wide usable potential window, slow electron-transfer kinetics in many systems, and, in some cases, electrocatalytic activity [14]. Among carbon-based electrodes, carbon paste electrodes (CPEs) are among the most widely used, as they allow modification not only at the surface but also within the bulk composition during preparation. Various organic and inorganic modifiers can be incorporated to tailor their electrochemical properties [15].

MATERIALS AND METHODS

Polymeric nanoparticles in the presence of favipiravir, used as the template (target) molecule, were synthesized via precipitation polymerization. Briefly, 0.4 mmol of the target molecule was dissolved in 20 mL of chloroform in a glass vessel, followed by the addition of 2 mmol of methacrylic acid monomer. The mixture was stirred using a mechanical stirrer for 1 h to allow the formation of a monomer–template pre-complex. Subsequently,

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8 mmol of ethylene glycol dimethacrylate as a cross-linker and 0.05 g of azobisisobutyronitrile (AIBN) as the radical initiator were added to the solution. To remove dissolved oxygen, nitrogen gas was purged through the solution for 10 min. The vessel was then tightly sealed and placed in an oil bath. The temperature was gradually increased to 60 °C and maintained for 24 h to complete the polymerization process [16]. After completion, the reaction vessel was cooled to room temperature and opened to allow evaporation of excess solvent. For the preparation of the carbon paste electrode (CPE), 25 mg of the binder (icosane) was placed in a watch glass, followed by the addition of 0.075 g of graphite powder. The components were thoroughly mixed until a homogeneous paste was obtained. The prepared paste was then packed into a glass syringe, and the electrode surface was polished on a suitable abrasive paper to obtain a smooth and reproducible surface. A copper wire was inserted into the paste during packing to ensure electrical contact.

For the fabrication of the modified carbon paste electrode, 10 mg of the modifying agent and 65 mg of graphite powder were first mixed in a glass mortar. The resulting mixture was then blended with 25 mg of binder to form a uniform paste. The prepared paste was subsequently transferred into the electrode body and compacted. After preparation, the electrode surface was polished to achieve a smooth and reproducible finish [17]. After electrode fabrication, the surface was polished using appropriate paper to ensure a smooth and reproducible surface. The calibration curve was constructed under optimized conditions, and the validation parameters of the proposed method, including linear response range, sensitivity, limit of detection (LOD), limit of quantification (LOQ), selectivity, and repeatability, were evaluated. Statistical analyses, including analysis of variance (ANOVA) and t-test, were employed to assess significant differences between the obtained results.

This study was approved by the Ethics Committee of Mazandaran University of Medical Sciences under approval code IR.MAZUMS.REC.1400.677.

RESULTS AND DISCUSSION

The development of simple, cost-effective, and highly sensitive analytical methods for the determination of antiviral drugs is of considerable importance, as these compounds are typically present in complex biological matrices at trace levels [18]. In the present study, the use of an unmodified carbon paste electrode demonstrated that acceptable analytical performance can be achieved even without the incorporation of nanomaterials or chemical modifiers. This finding is particularly significant from a practical perspective, since many previously reported sensors require complex fabrication procedures, high cost, and often suffer from limited stability [19].

Electrochemical investigation of the studied drug on the carbon paste electrode revealed one anodic and one cathodic peak, indicating an irreversible redox behavior Fig 1.

The observed electrochemical behavior of favipiravir suggests that the pyrazine ring structure of the molecule plays a key role in the electron-transfer processes. The presence of various substituent groups attached to this ring may reduce the reversibility of the redox process, leading to the predominance of irreversible reaction pathways. This behavior is consistent with previous reports

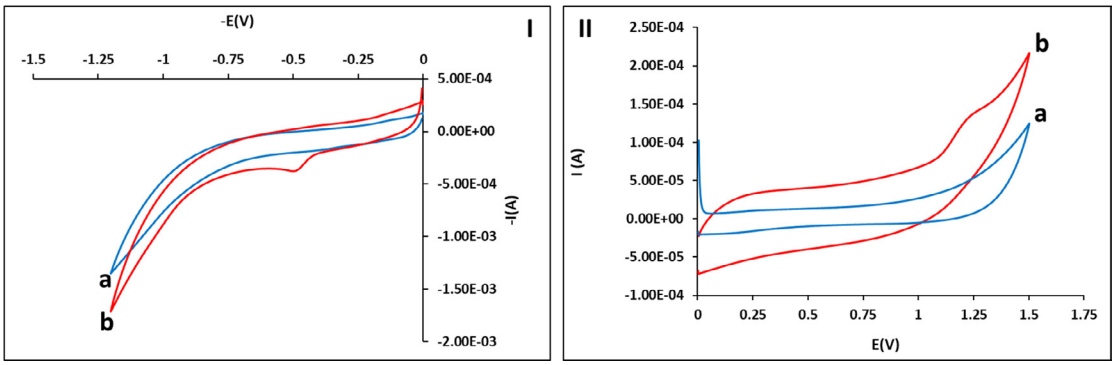


Fig. 1. Cathodic (I) and Anodic (II) electrochemical behavior of the on the carbon paste electrode in (a) supporting electrolyte and (b)1.0x10^{-4} M FAV

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on pyrazine derivatives, indicating that the nature of substituents, as well as environmental conditions such as pH and electrolyte composition, can significantly influence charge-transfer mechanisms (20). (Fig 2)

The study of pH effects showed that with increasing acidity, the peak potentials shifted toward more negative values, suggesting the involvement of protons in the reaction mechanism (Fig. 3). Moreover, the simultaneous involvement of protons and electrons in the oxidation and reduction processes indicates that the reactions proceed via coupled proton–electron transfer (CPET) mechanisms, which are commonly observed in many heteroaromatic compounds.

Evaluation of different supporting electrolytes demonstrated that the highest cathodic peak current was obtained under acidic conditions, and 0.1 M sulfuric acid was selected as the optimal medium. In contrast, for the anodic process, the peak current remained nearly constant within the pH range of 4–8, while a decrease was observed under strongly acidic conditions; therefore, pH 5 was selected as the optimal condition for the oxidation process.

Optimization of instrumental parameters indicated that the peak current is dependent on pulse amplitude, pulse time, and potential step. The

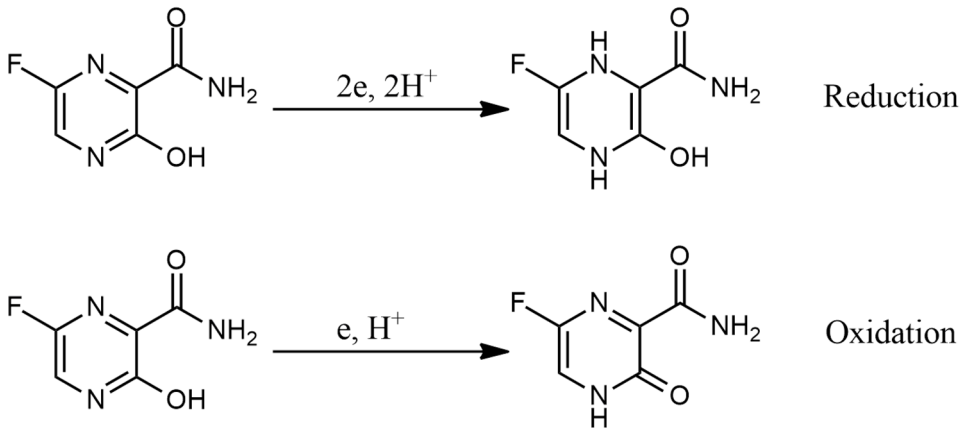


Fig. 2. Electrochemical oxidation and reduction reactions of favipiravir at the electrode surface.

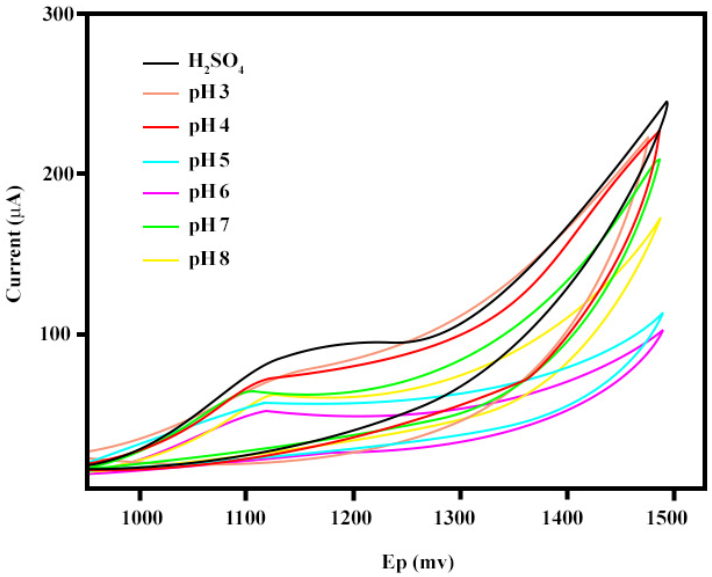


Fig. 3. A) Cyclic voltammetry plots for pHs 3-8.

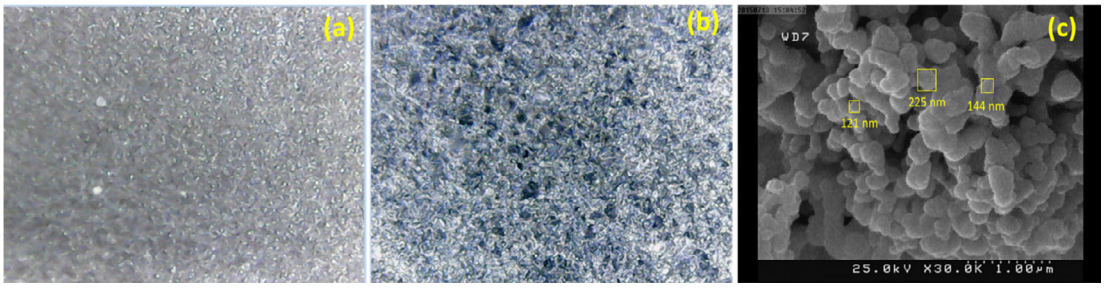


Fig. 4. Images of (a) unmodified and (b) modified electrode surface. (c) FE-SEM image of Polymeric particles.

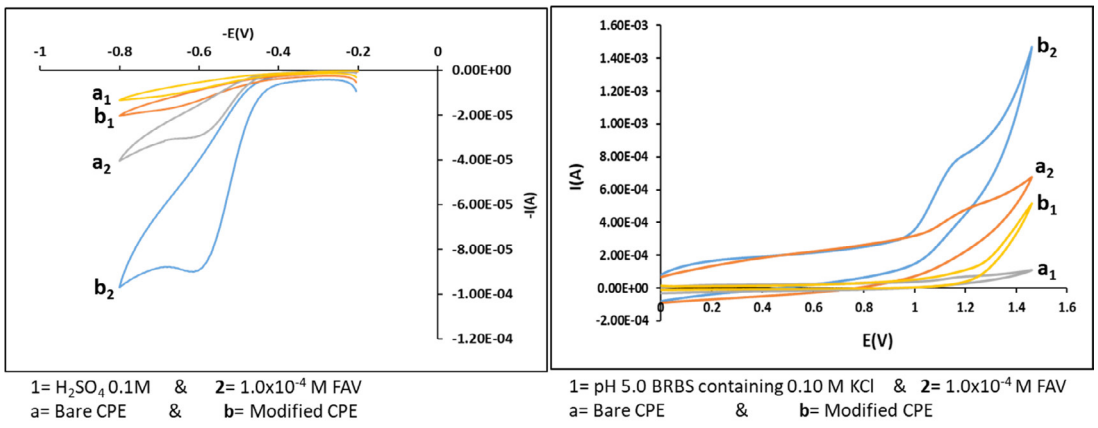


Fig. 5. Reduction (Left) and oxidation (right) CVs of unmodified (a_1 & a_2) and modified (b_1 & b_2) carbon paste electrodes in supporting electrolyte and $1.0x10^{-4}$ M FAV.

results showed that, for the reduction process, the maximum peak current was obtained at a pulse amplitude of 120 mV, a pulse time of 1 ms, and a potential step of 8 mV. For the oxidation process, the highest response was achieved at a pulse amplitude of 120 mV, a pulse time of 3 ms, and a potential step of 8 mV. These findings indicate that the oxidation process is more sensitive to variations in pulse time. The dependence of the electrochemical response on diffusion suggests that mass transport of the drug molecule in solution is the dominant factor governing the current response. This may indicate that the adsorption interaction between the drug molecule and the electrode surface is relatively weak [21]. Such be havior is advantageous in analytical applications, as limited surface adsorption generally enhances repeatability and minimizes electrode fouling or memory effects. In addition, diffusion-controlled behavior suggests that the method can operate over a wide concentration range without surface saturation effects.

Surface images of the of (a) unmodified CPE, (b) modified CPE and also (c) polymeric materials (modifier) are shown in Fig. 4. As can be seen, the synthesized polymer materials have nano and micro dimensions, and their use in the electrode composition causes porosity and increases its surface area, as well as increases the surface adsorption of the drug due to the functional groups present in the polymer structure. The combination of these factors has increased the response of the modified electrode to the drug.

The response of bare CPE and modified CPE to the Favipiravir was also compared (Figure 5). As can be seen, the use of the modifier in the electrode composition resulted in a significant improvement in the sensor response, which is due to the advantages mentioned above.

Analytical performance studies demonstrated a strong linear relationship between the cathodic peak current and drug concentration (Fig. 6) over the range of 1–600 µM. The calibration equation exhibited a high correlation coefficient ($R^2 = 0.996$),

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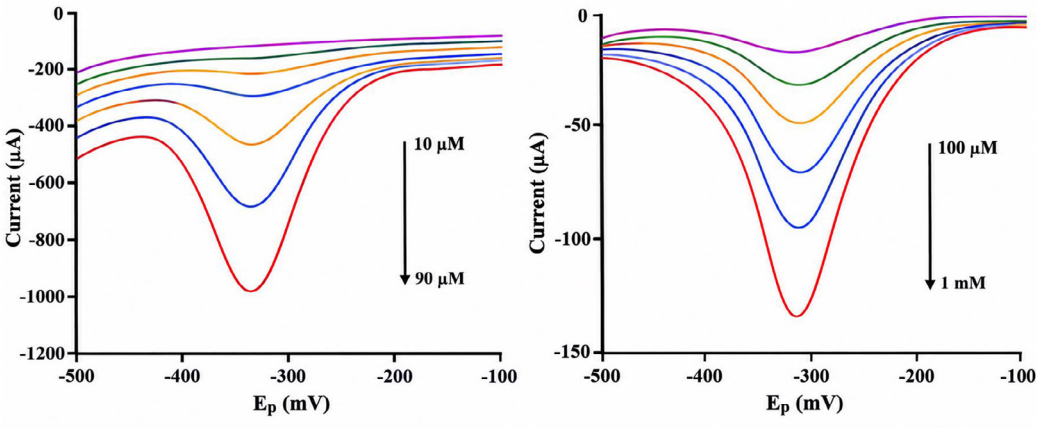


Fig. 6. Cathodic voltammetric signals at different concentration ranges (10–90 µM and 100 µM–1 mM).

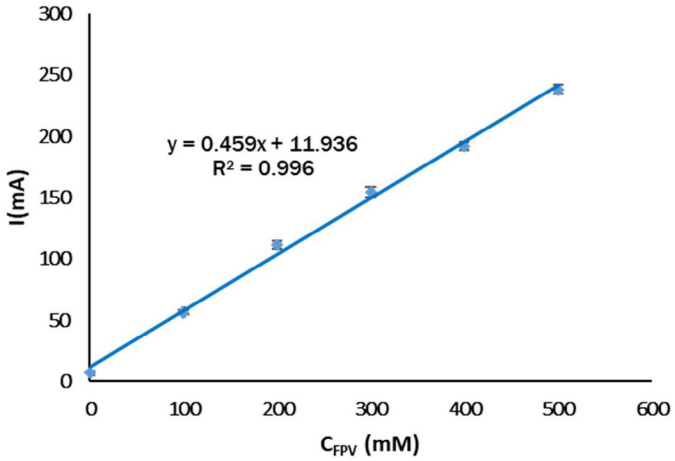


Fig. 7. Calibration plot of current versus drug concentration (1–600 µM).

indicating excellent accuracy and linearity within this concentration range (Fig. 7). For the anodic process, two linear dynamic ranges were observed. In the range of 5–200 µM, the slope was 0.626 with a correlation coefficient of 0.993. In the higher concentration range (200–600 µM), a decrease in slope was observed, with a correlation coefficient of 0.992. This reduction in sensitivity at higher concentrations may be attributed to mass transport limitations or partial saturation of the electrode surface.

The limits of detection (LOD) for the proposed sensor was calculated according to relationship of $3S_b/m$ where S_b is represented the standard deviation of the peak current for the blank solution (n= 7), and m is the slope of the calibration curve.

Based on the equation, the detection limits were calculated as 0.35 µM for the reduction process and 1.55 µM for the oxidation process, indicating higher sensitivity for the cathodic pathway.

Moreover, the sensitivity values were 8302 and 3939 µA mM^{-1} cm^{-2} for the reduction and oxidation processes, respectively, confirming approximately two-fold higher sensitivity for the reduction route (Fig. 5).

Compared with electrochemical sensors based on nanostructured materials, although enhanced sensitivity has been reported in some cases, their complex fabrication procedures, high cost, and often reduced reproducibility can limit their practical applicability. In contrast, the use of a simple commercial electrode provides a favorable balance between analytical performance and operational feasibility [23]. This advantage becomes particularly important in situations requiring rapid and low-cost measurements. Accuracy and precision

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studies demonstrated satisfactory performance of the proposed method, with mean recovery values close to 100% and low data dispersion. The relative standard deviation (RSD) of the calibration slope was 3.45% for the reduction process and 5.13% for the oxidation process, indicating good repeatability. The limits of quantification were calculated as 1.15 μM for the reduction process and 5.11 μM for the oxidation process. Interference studies showed that most common biological species did not significantly affect the analytical signal. Simple sugars, urea, and inorganic ions had negligible effects on the cathodic current even at high excess levels. Ascorbic acid and dopamine were tolerated up to a 250-fold excess, copper ions up to a 10-fold excess, and uric acid at equimolar concentration, demonstrating the good selectivity of the method in complex biological matrices.

From a selectivity standpoint, the resistance of the method to interference from common biological species indicates that the electrochemical oxidation/reduction of favipiravir occurs at potentials that are well separated from those of most endogenous electroactive compounds [22]. This feature is particularly important for real sample analysis, where numerous electroactive species may coexist and potentially cause analytical errors. Therefore, the ability to discriminate the drug signal from other matrix components confirms the suitability of the method for bioanalytical applications. Application of the method to real samples indicated that the drug content in a commercial tablet was 215 mg per tablet, showing minimal deviation from the labeled value of 200 mg and confirming the accuracy of the method. Recovery studies in spiked samples showed recoveries of 98.9%, 106%, and 101% for the cathodic process and 102%, 102.1%, and 104% for the anodic process at spiked concentrations of 100, 150, and 200 μM, respectively. In synthetic serum samples, recoveries were approximately 103% for the reduction process and 104% for the oxidation process, confirming the reliability of the method in biological matrices.

In terms of practical application, the successful direct determination of the drug in pharmaceutical and biological samples demonstrates that matrix effects are limited under the proposed conditions. This may be attributed to the stability of the electrochemical signal under different environmental conditions, as well as the appropriate selection of the supporting electrolyte. Such characteristics are highly valuable for the development of rapid pharmaceutical screening and quality control methods. The results demonstrate that the proposed method offers a wide linear range, low detection limits, high accuracy, good repeatability, and excellent selectivity. In addition, the simplicity of electrode fabrication, low cost, and absence of surface modification steps represent major advantages, making the method suitable for routine determination of this drug in pharmaceutical and biological samples. The results of this study demonstrate that the design of analytical methods based on simple electrode systems can serve as an effective strategy for the development of practical and accessible pharmaceutical sensors. Furthermore, a deeper understanding of charge-transfer mechanisms in pharmaceutical compounds may contribute to the design of a new generation of more selective and stable electrochemical sensors.

CONCLUSION

The results of this study demonstrated that an electrochemical sensor based on a carbon paste electrode can serve as an effective analytical platform for the determination of favipiravir in pharmaceutical and biological samples. The satisfactory performance of the sensor in terms of sensitivity, selectivity, repeatability, and linear dynamic range indicates that acceptable analytical responses can be achieved even without the use of complex surface modifiers or expensive nanomaterials. This finding is particularly important from an application perspective, as the simplicity of electrode fabrication, low cost of consumables, and lack of requirement for sophisticated instrumentation enable broad applicability in routine laboratories and even in portable analytical systems. Validation results, including a low detection limit, near-quantitative recovery values, and satisfactory resistance to interference from common biological species, confirm the applicability of the proposed method for real sample analysis. Overall, the developed approach can be considered a suitable alternative to more complex instrumental techniques for drug monitoring. In addition, the findings of this study may facilitate the development of simple, low-cost, and portable electrochemical sensors for bioanalytical and pharmaceutical applications, while also providing a foundation for future studies aimed at designing more selective and stable sensing platforms.

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CONFLICTS OF INTEREST

The authors declare that there are no conflicts of interest.

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