



Original Article

Voltammetric Sensor for Acyclovir Determination Based on MoSe₂/rGO Nanocomposite Modified Electrode

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ABSTRACT

In this study, MoSe₂/rGO nanocomposite modified screen printed graphite electrode (SPGE) was designed for acyclovir (ACV) determination. The electrochemical investigation and measurement of ACV were performed by applying some voltammetric techniques and chronoamperometry. After modification of SPGE, the enhancement of the voltammetric response and the reduction of overpotential of ACV confirmed the good electrocatalytic ability of MoSe₂/rGO/SPGE sensor towards the ACV oxidation. The voltammetric method (differential pulse voltammetry (DPV)) was used to investigate the determination ability of MoSe₂/rGO nanocomposite/SPGE towards ACV determination under the optimum parameters and conditions. The MoSe₂/rGO/SPGE sensor indicated appreciable sensing ability towards ACV, with an optimal linear response from 0.03-190.0 μM and a limit of detection (LOD) of 0.01 μM. More importantly, the practical applicability of the designed sensor was confirmed in the ACV quantification in ACV tablet and urine samples, showing its potential application for real sample analysis.

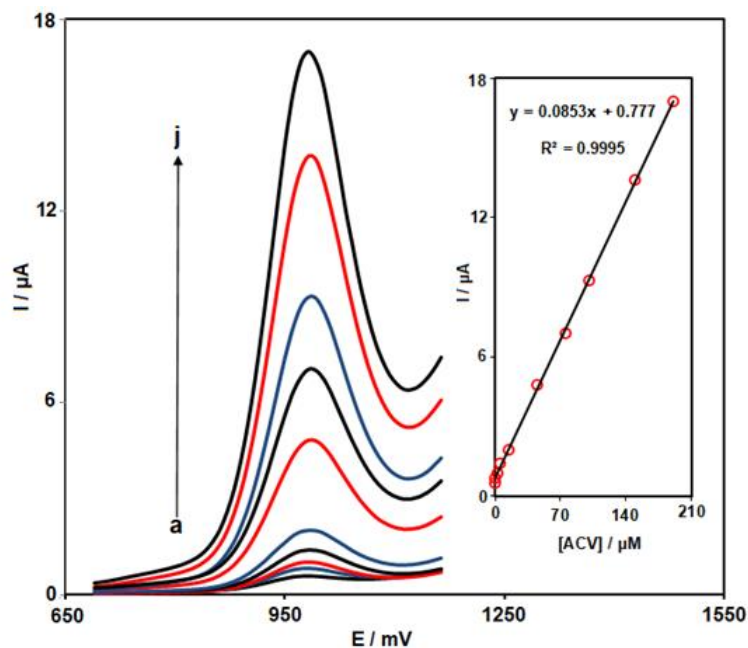
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GRAPHICAL ABSTRACT



A novel electrochemical sensor for determination of acyclovir was constructed

Introduction

In last years, the swift emergence of viral diseases has been recognized as a serious threat to both human and veterinary health [1]. More recently, the viral diseases surpassed other infectious diseases to become the leading cause of death worldwide. This evolution has made viral diseases a paramount concern for public health. Acyclovir (ACV) is known as a synthetic nucleoside analog of purine derived from guanine. However, it differs from guanine in that it lacks a 3'-hydroxyl on its side chain. It has been extensively utilized in the clinical treatment of various viral diseases, including Epstein-Barr virus, varicella-zoster virus (VZV), hepatitis B virus (HBV), and herpes simplex virus (HSV). This medication has demonstrated remarkable therapeutic benefits in treating viral diseases such as cold sores, encephalitis, infections of central nervous system, keratitis, and corneal blindness [2,3]. When present in high concentrations in the body, ACV can cause adverse effects such as nausea and diarrhea. In addition, high doses of this medication can potentially cause serious side effects related to the kidneys and low platelet counts [4,5].

Therefore, the necessity of using a powerful analytical tool with high selectivity and sensitivity, and quick response is clearly evident for ACV determination in pharmaceutical compounds and biological samples. So far, several analytical techniques have been reported for the ACV detection, including spectrophotometry [6], radioimmunoassay [7], capillary electrophoresis [8], and chromatography [9,10], and. Despite being well-established and widely accepted for detecting ACV, these methods often encounter limitations such as expensive equipment, extensive sample preparation, the need for specialized expertise, and time-consuming procedures. These factors can render these methods unsuitable for routine analysis in clinical settings and can restrict their availability to healthcare professionals and patients. Due to their sensitivity, simplicity, rapid response and inexpensive instrumentation, electrochemical methods based on chemically modified electrodes (CMEs) present a promising alternative [11-21].

Research indicates that the screen-printing technology commonly employed in microelectronics holds significant value in producing electrodes for disposable

electrochemical (bio) sensors. Screen-printed electrodes (SPEs) offer a range of benefits such as simplified operation, adaptability, cost-effectiveness, portability, reliability, reduced dimensions, and scalability for mass production [22,23]. Consequently, it finds extensive utility within the realm of electroanalytical chemistry. Furthermore, the implementation of an SPE eliminates the need for meticulous cleaning procedures that traditional electrodes, such as glassy carbon electrodes, require. This innovation addresses the limitations inherent in conventional electrode systems, which necessitate frequent recalibration, lack stability, and prove unsuitable for on-site analyses due to their lengthy completion times spanning several hours. Furthermore, traditional electrode systems mandate the expertise of skilled professionals due to their complex isolation and washing protocols. Consequently, the shortcomings associated with traditional electrode systems have rendered them less effective compared to the advantages offered by SPEs. Nanotechnology, as one of the top advances of recent decades, has rapidly become one of the most important research and application fields in various domains [24-29]. More importantly, nanotechnology has a huge potential in the design, fabrication, and development of novel and practical sensors [30-37]. With the development of the nanostructures application in electrochemical sensors, it is known that the use of nanostructures to modify the surface of the electrode leads to the improvement of the speed of the electron transfer process, reduction of overvoltage and increase of the efficiency of the electrode [38-43].

Recently, there has been significant attention in research towards molybdenum-based two-dimensional transition metal dichalcogenides (2D nanomaterials) owing to their amazing physical and chemical attributes such as substantial surface area, heightened electronic conductivity, remarkable specific capacitance, their layered structure, inexpensive price, outstanding electrocatalytic activity, and good chemical stability [44,45].

Typically, chalcogenides like MoX_2 (where, X = S and Se) are synthesized to exhibit a two-dimensional layered configuration similar to graphite. The individual MoX_2 layer comprises a covalently bonded arrangement of Mo elements with chalcogenide elements. These layers are held together through gentle interactions (Van der Waals). MoSe_2 surpasses MoS_2 in terms of electrical conductivity and electrocatalytic performance due to its elevated metallic attributes and the presence of actively catalytic unsaturated selenium edges [46-48]. Nonetheless, the inert nature of the exposed planes on MoSe_2 nanosheets limits their reactivity, as the active sites crucial for catalysis are primarily concentrated along the unsaturated selenium edges. Therefore, due to the sparse distribution of these active edge sites, the overall electrocatalytic activity of MoSe_2 remains unsatisfactory. To tackle these limitations, MoSe_2 has been employed in conjunction with highly conductive carbonaceous materials, resulting in an enhancement of both MoSe_2 's conductivity and electrocatalytic activity. Because of its exceptional capacity to enhance electron transfer and its extensive surface area, reduced graphene oxide (rGO) can be readily combined with two-dimensional nanosheets like MoSe_2 to create heterostructures [49,50].

Concerning the aforementioned factors, the present investigation introduces the utilization of a novel and disposable sensor for the detection of ACV. This sensor capitalizes on the enhancement of the SPGE surface through the incorporation of a MoSe_2/rGO nanocomposite. Notably, the MoSe_2/rGO nanocomposite exhibited good electrocatalytic performance towards the ACV determination. This was evidenced by its notably narrow LOD, impressive sensitivity, and minimal over-voltage requirements. The resultant sensor was effectively employed for the quantification of ACV in urine samples and ACV tablets.

Experimental

Equipments and Reagents

The PGSTAT 302N Autolab (Metrohm, the Netherlands) operated by GPES software and connected to personal PC was applied to perform all electrochemical experiments. The pH control of phosphate buffer solution (PBSs) was

measured by using a Metrohm 713 pH meter. The analytical grade of reagents with high purity were used in the present work as provided from Merck and Sigma-Aldrich companies without any further processing. The synthesis and characterization of MoSe₂/rGO nanocomposite has been given in our previously reported work [51]. Figure 1 displays its FE-SEM image.

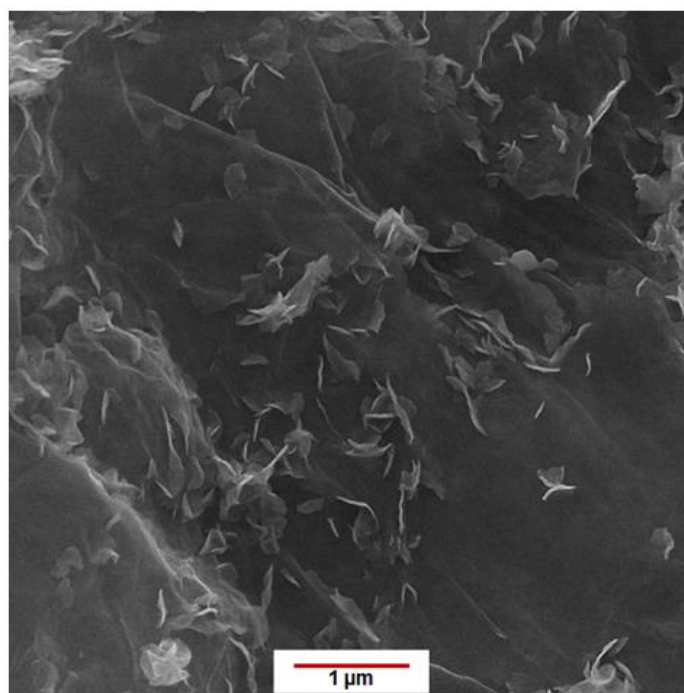


Figure 1: FE-SEM image of MoSe₂/rGO nanocomposite

Preparation of the MoSe₂/rGO Nanocomposite Modified SPGE

The procedure for SPGE modification is as follow: 1.0 mg of MoSe₂/rGO was initially subjected to ultrasonication for at least 20 min in 1 mL of solvent to prepare MoSe₂/rGO suspension, and then MoSe₂/rGO suspension (4.0 μL) was dropped carefully on the SPGE surface. After drying at room temperature, the MoSe₂/rGO/SPGE sensor was prepared.

The surface areas of unmodified SPGE and MoSe₂/rGO/SPGE were calculated to determine the effect of modification process. Accordingly, the CVs at various scan rates were recorded for 0.1 M KCl solution containing 1.0 mM K₃[Fe(CN)₆]. The Randles-Sevcik equation was used to calculate the surface areas. The

MoSe₂/rGO/SPGE demonstrated an effective surface area (0.113 cm²), which was 3.6 times greater than the surface area on unmodified electrode.

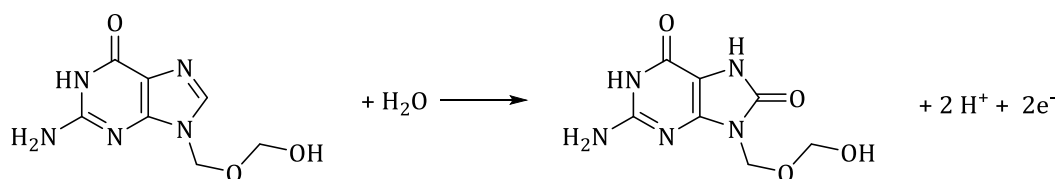
Results and Discussion

Investigating the Performance of the MoSe₂/rGO on the ACV Determination

The pH of buffer solution is a key parameter in electroanalysis of compounds. Therefore, the effect of pH in the present work was investigated in PBS (0.1 M) with various values of pH from 4.0 to 9.0 containing 75.0 μM ACV at MoSe₂/rGO/SPGE. From the voltammograms, the current responses of ACV enhanced with the increasing in pH value from 4.0 to 7.0, and then decreased at pH values higher than 7.0.

Therefore, the selected optimal buffer solution is PBS 0.1 M at pH 7.0 due to the highest peak

current of ACV in this pH. The oxidation mechanism of ACV is presented in [Scheme 1](#).



Scheme 1: The oxidation mechanism of ACV

To confirm the electrocatalytic response of prepared sensor ($\text{MoSe}_2/\text{rGO}/\text{SPGE}$) towards the electrochemical reaction of ACV, the CV responses of $100.0\ \mu\text{M}$ ACV in PBS (0.1 M-pH 7.0) were recorded at various electrodes (unmodified SPGE ([Figure 2](#) (voltammogram a)) and $\text{MoSe}_2/\text{rGO}/\text{SPGE}$ ([Figure 2](#) (voltammogram b)) at a scan rate of $50\ \text{mV}\cdot\text{s}^{-1}$. From the obtained voltammograms, it is obvious that the observed electrochemical process is an irreversible process, because only one well-defined oxidation peak was obtained due to the ACV presence. Also,

a weak oxidation peak with low I_{pa} was observed for ACV at bare SPGE (voltammogram a). In contrast, the SPGE surface modified with a nanocomposite of MoSe_2/rGO (voltammogram b) exhibited a notably amplified $I_{\text{pa}} = 9.35\ \mu\text{A}$ and a shifted E_{pa} toward negative values ($E_{\text{pa}} = 990\ \text{mV}$) in the presence of ACV, compared to the bare SPGE. This prominent enhancement in the oxidation peak characteristics can be ascribed to the remarkable impacts of the MoSe_2 and rGO sheets and their synergistic effects in the ACV oxidation.

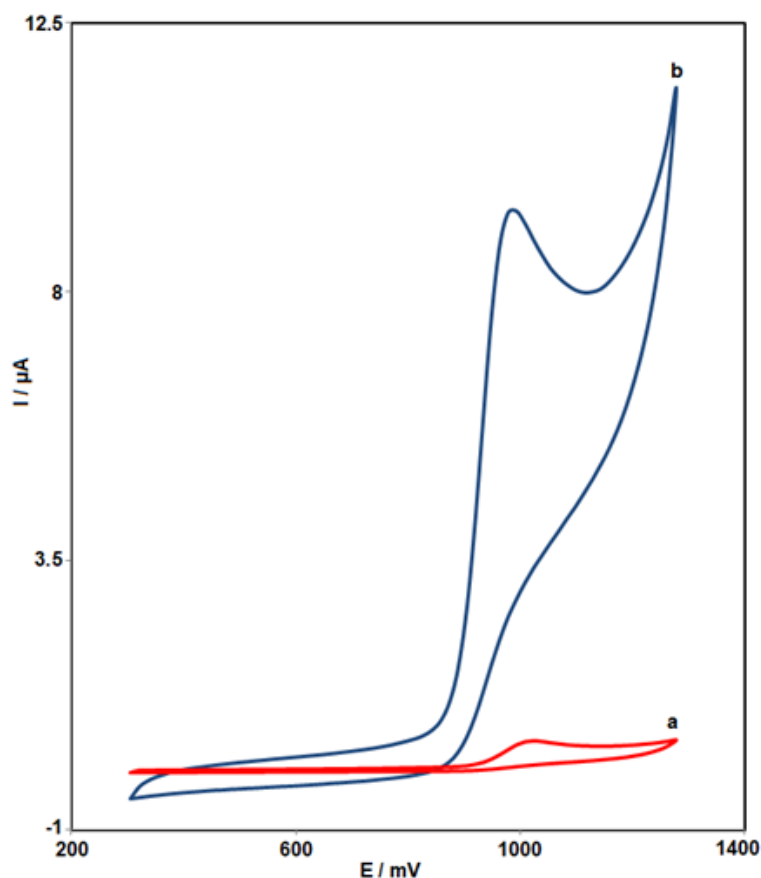


Figure 2: CVs of bare SPGE (a) and $\text{MoSe}_2/\text{rGO}/\text{SPGE}$ (b) in 0.1 M PBS at pH 7.0 containing $100.0\ \mu\text{M}$ ACV at $50\ \text{mV}\ \text{s}^{-1}$ scan rate

Effect of Scan Rate

In addition, the effect of scan rate (ν) on ACV oxidation was evaluated using LSV on the MoSe₂/rGO/SPGE in PBS (0.1 M at pH 7.0) containing 90.0 μM ACV at various scan rates (10 to 300 $\text{mV}\cdot\text{s}^{-1}$) (Figure 3). Figure 3 illustrates that the I_{pa} of ACV was increased with increasing of

scan rates. Moreover, the data presented in Inset of Figure 3 shows a strong linear dependence between the I_{pa} and the $\nu^{1/2}$ within the range of 10-300 $\text{mV}\cdot\text{s}^{-1}$ ($I_{pa} (\mu\text{A}) = 1.6473\nu^{1/2} (\text{mV s}^{-1})^{1/2} - 2.2396$ ($R^2 = 0.9991$)), which suggests a diffusion-controlled process on the MoSe₂/rGO/SPGE surface.

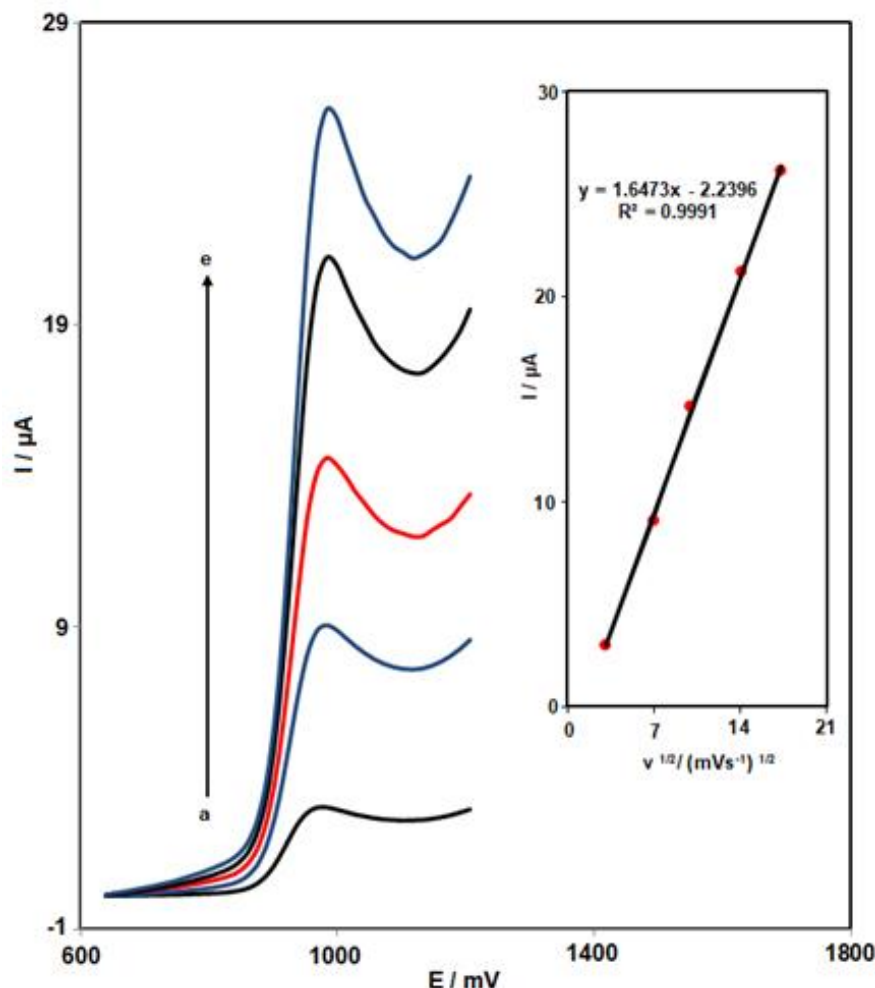


Figure 3: LSVs of MoSe₂/rGO/SPGE in 0.1 M PBS at pH 7.0 containing 90.0 μM ACV at different scan rates ((a) 10, (b) 50, (c) 100, (d) 200, and (e) 300 mV s^{-1}). Inset: the corresponding plot of I_{pa} (μA) vs. $\nu^{1/2}$ (mV s^{-1})^{1/2}

Chronoamperometric Measurements of ACV

A chronoamperometric investigation was conducted to determine the diffusion coefficient (D) of ACV at the MoSe₂/rGO/SPGE. The results of this investigation are depicted in Figure 4, which illustrates the obtained chronoamperograms for ACV at various concentrations in a pH 7.0 PBS. The reaction of an electroactive substance with a D is described by Cottrell's equation when the process is

constrained by mass transport. In Figure 4A, a linear correlation is obtained between the current (I) and the square root of time ($t^{1/2}$) for the oxidation of varying ACV concentrations. The slopes obtained from the linear fits were then correlated with the different ACV concentrations, as demonstrated in Figure 4B. By utilizing the plotted slope in conjunction with the Cottrell equation, the D of ACV was calculated to be $2.1 \times 10^{-5} \text{ cm}^2/\text{s}$.

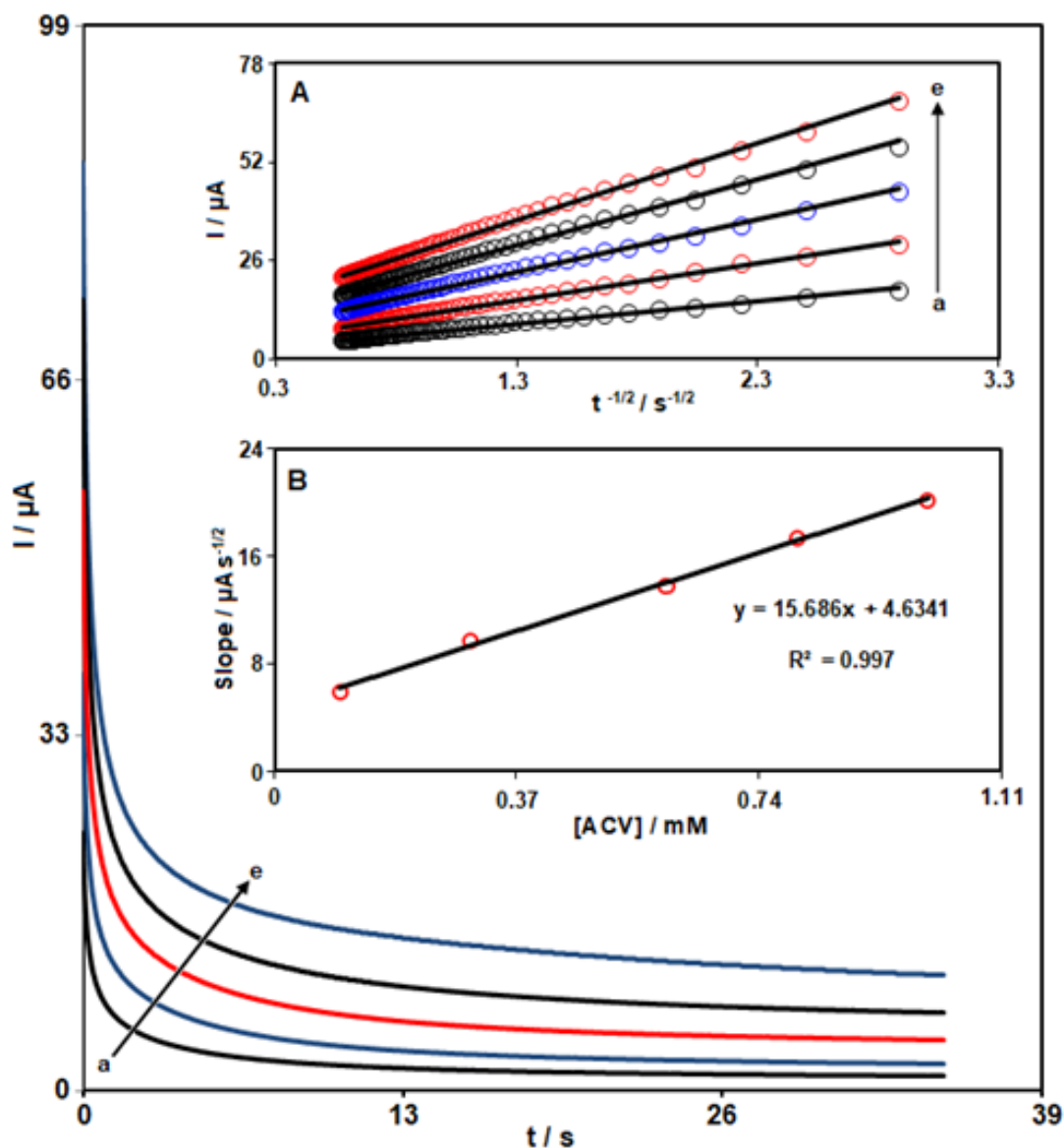


Figure 4: Chronoamperometric responses of MoSe₂/rGO/SPGE in 0.1 M PBS at pH 7.0 containing ACV at different concentrations ((a) 0.1), (b) 0.3), (c) 0.6), (d) 0.8), and (e) 1.0 mM) of ACV). Insets: corresponding plots of I (μA)- $t^{1/2}$ ($\text{s}^{-1/2}$) curves from the chronoamperograms (A) and corresponding plots of obtained slopes-[ACV]

Quantitative Analysis of ACV by DPV

Figure 5 demonstrates the DPV responses for ACV at different concentrations in PBS (0.1 M-pH 7.0) at the MoSe₂/rGO/SPGE in the following conditions: step potential (0.01 V) and pulse amplitude (0.025 V). It was found that the I_{pa} of ACV increased proportionally along with increasing in ACV concentration over a range of

0.03 μM to 190.0 μM . Likewise, by plotting the I_{pa} of ACV vs. its concentrations a good linearity was obtained (I_{pa} (μA) = 0.0853 C_{ACV} (μM) + 0.777 ($R^2 = 0.9995$)) (Figure 5-Inset). The LOD value was obtained to be 0.01 μM . In addition, the performance of MoSe₂/rGO/SPGE sensor was compared with some of reported electrochemical sensors for ACV determination (Table 1).

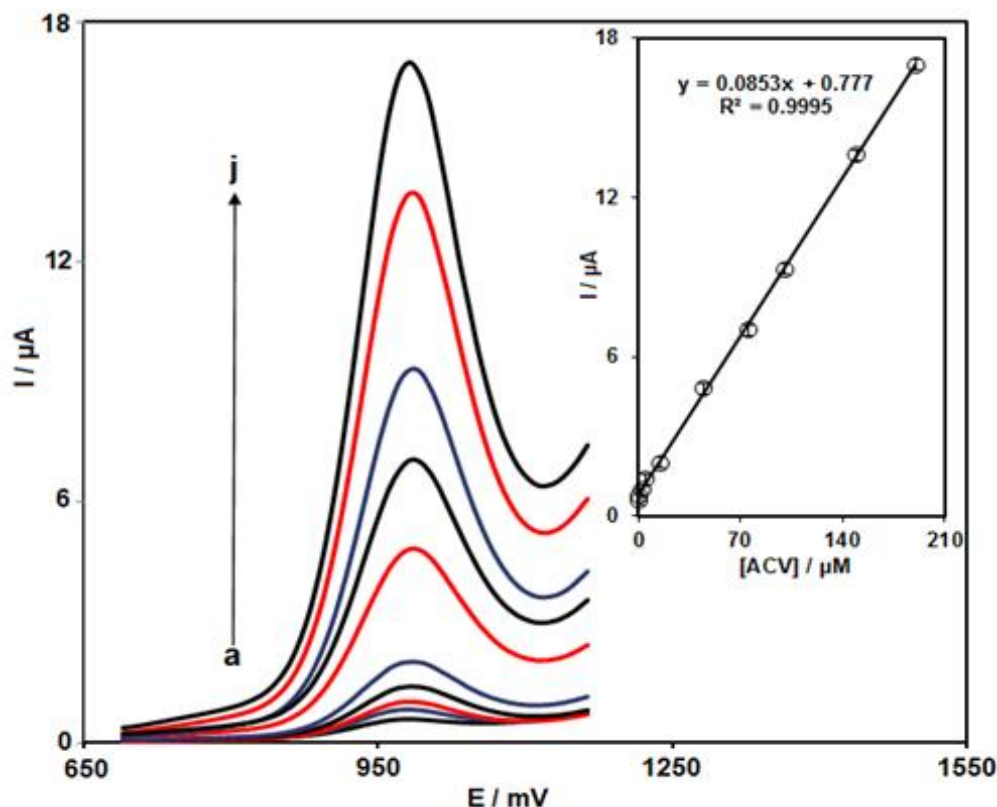


Figure 5: DPVs of MoSe₂/rGO/SPGE in 0.1 M PBS at pH 7.0 containing various concentrations of ACV ((a) 0.03, (b) 0.3, (c) 2.5, (d) 5.0, (e) 15.0, (f) 45.0, (g) 75.0, (h) 100.0, (i) 150.0, and (j) 190.0 μM). Inset: The linear plot of I_{pa} (μA) vs. C_{ACV} (μM)

Table 1: The performance of MoSe₂/rGO/SPGE sensor in comparison with some of previous reported electrochemical sensors for ACV determination

Electrochemical Sensor	Electrochemical Method	Linear Range	LOD	Ref.
Ca-doped ZnO nanoparticles (NPs)/GCE	Square wave voltammetry (SWV)	8.0×10 ⁻⁸ -2.4×10 ⁻⁵ M	6.18 nM	[11]
Reduced graphene oxide (rGO)-TiO ₂ -Au nanocomposite/glassy carbon electrode (GCE)	Linear sweep voltammetry (LSV)	1-100 μM	0.3 μM	[52]
Magnetic CdO NPs/carbon paste electrode (CPE)	DPV	1-100 μM	300 nM	[53]
Multiwalled carbon nanotube/iron-doped polypyrrole/GCE	LSV	0.03-10.0 μM	10.0 nM	[54]
Fullerene-C ₆₀ /GCE	DPV	9.0×10 ⁻⁸ -6.0×10 ⁻⁶ M	14.8 nM	[55]
MoSe ₂ /rGO/SPGE	DPV	0.03-190.0 μM	0.01 μM	This work

Stability Studies of MoSe₂/rGO/SPGE for ACV Determination

The stability studies of MoSe₂/rGO/SPGE sensor were done by recording the voltammetric response of this sensor towards 60.0 μM ACV over 12 days. The obtained results demonstrated

that the voltammetric response retained 97.1% of its initial response after 12 days, indicating the good stability of the developed sensor.

Analytical Application of MoSe₂/rGO/SPGE for Determination of ACV in Real Samples

The urine samples and ACV tablets were used to investigate the practical applicability of the MoSe₂/rGO/SPGE for ACV determination by DPV. The recovery studies were done by standard

addition method to confirm the accuracy by spiking the ACV tablet and urine samples with ACV in various concentrations (Table 2). The recovery values varied from 96.7% to 104.2%. Also, the results showed a good precision as can be inferred from the low values of RSD (%) obtained (n = 5).

Table 2: The determination results of ACV in urine samples and ACV tablets

Real sample	Added concentration in μM	Detected concentration in μM	Recovery (%)	R.S.D. (%)
ACV tablet	0	3.2	-	2.4
	2.0	5.1	98.1	3.3
	4.0	7.5	104.2	1.7
	6.0	9.1	98.9	2.6
	8.0	11.3	100.9	2.1
Urine	0	-	-	-
	5.0	5.1	102.0	2.4
	7.0	6.9	98.6	3.0
	9.0	8.7	96.7	2.0
	11.0	11.1	100.9	2.7

Conclusion

In the presented study, a facile and novel MoSe₂/rGO-modified SPGE was designed and applied for sensitive and accurate determination of ACV. With the synergistic effect from MoSe₂ and rGO sheets, the designed sensor exhibited good performance for oxidation of ACV by reducing the over-potential and enhancing the current response. In addition, the MoSe₂/rGO/SPGE sensor exhibited analytical performances for determining ACV, including wide response range (0.03 μM to 190.0 μM), low LOD (0.01 μM), and high sensitivity (0.0853 $\mu\text{A}\cdot\mu\text{M}^{-1}$). Also, MoSe₂/rGO/SPGE sensor demonstrated good stability for ACV determination. Finally, the designed sensor was successfully utilized for ACV determination in ACV tablet and urine samples, with high reliability and accuracy.

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